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(54) Title: NUCLEIC ACIDS AND PROTEINS FROM GROUP B STREPTOCOCCUS

(57) Abstract: Novel protein antigens from Group B Streptococcus are described, together with the nucleic acid sequences encoding them. The use of vaccines and screening methods is also described.

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### Proteins

The present invention relates to proteins derived from *Streptococcus agalactiae*, nucleic acid molecules encoding such proteins, and the use of the proteins as  
5 antigens and/or immunogens and in detection/diagnosis. It also relates to a method for the rapid screening of bacterial genomes to isolate and characterise bacterial cell envelope associated or secreted proteins.

The Group B Streptococcus (GBS) (*Streptococcus agalactiae*) is an encapsulated  
10 bacterium which emerged in the 1970s as a major pathogen of humans causing sepsis and meningitis in neonates as well as adults. The incidence of early onset neonatal infection during the first 5 days of life varies from 0.7 to 3.7 per 1000 live births and causes mortality in about 20% of cases. Between 25-50% of neonates surviving early onset infections frequently suffer neurological sequelae. Late onset neonatal  
15 infections occur from 6 days to three months of age at a rate of about 0.5 - 1.0 per 1000 live births.

There is an established association between the colonisation of the maternal genital tract by GBS at the time of birth and the risk of neonatal sepsis. In humans it has  
20 been established that the rectum may act as a reservoir for GBS. Susceptibility in the neonate is correlated with the a low concentration or absence of IgG antibodies to the capsular polysaccharides found on GBS causing human disease. In the USA strains isolated from clinical cases usually belong to capsular serotypes Ia, Ib, II, III although serotype V may be of increasing significance. Type VIII GBS is the major  
25 cause of neonatal sepsis in Japan.

A possible means of prevention involves intra or postpartum administration of antibiotics to the mother but there are concerns that this might lead to the emergence

of resistant organisms and in some cases allergic reactions. Vaccination of the adolescent females to induce long lasting maternally derived immunity is one of the most promising approaches to prevent GBS infections in neonates. The capsular polysaccharide antigens of these organisms have attracted most attention as with regard to vaccine development. Studies in healthy adult volunteers have shown that serotype Ia, II and III polysaccharides are non-toxic and immunogenic in approximately 65%, 95% and 70% of non-immune adults respectively. One of the problems with using capsule antigens as vaccines is that the response rates vary according to pre-immunisation status and the polysaccharide antigen and not all vaccinees produce adequate levels of IgG antibody as indicated in vaccination studies with GBS polysaccharides in human volunteers.

Some people do not respond despite repeated stimuli. These properties are due to the T-independent nature of polysaccharide antigens. One strategy to enhance the immunogenicity of these vaccines is to enhance the T cell dependent properties of polysaccharides by conjugating them to a protein. The use of polysaccharide conjugates looks promising but there are still unresolved questions concerning the nature of the carrier protein. A conjugate vaccine against GBS would require at least 4 different conjugates to be prepared adding to the cost of a vaccine.

Approaches to vaccination against GBS infections which rely on the use of capsular polysaccharides have the disadvantage that response rates are likely to vary considerably according to pre-immunisation status and the particular type of polysaccharide antigen used. Results of trials with conjugate vaccines in human volunteers have indicated that response rates may only be around 65% for some of the key capsule antigens (Larsson *et al.*, *Infection and Immunity* 64:3518-3523 (1996)). It is also not clear whether all individuals responding to the vaccine would have adequate levels of polysaccharide specific IgG which can cross the placenta and

afford immunity to neonates. By conjugating a protein carrier to the polysaccharide antigen it may be possible to convert them to T-cell dependent antigens and enhance their immunogenicity.

5 Preliminary studies with GBS type III polysaccharide-tetanus toxoid conjugate have been encouraging (Baker *et al.*, *Reviews of Infectious Diseases* 7:458-467 (1985), Baker *et al.*, *The New England Journal of Medicine* 319:1180-1185 (1988), Paoletti *et al.*, *Infection and Immunity* 64:677-679 (1996), Paoletti *et al.*, *Infection and Immunity* 62:3236-3243 (1994)) but in developed countries the use of tetanus may be  
10 disadvantageous since most adults will have been immunised against tetanus within the past five years. Additional boosters with tetanus toxoid may cause adverse reactions (Boyer., *Current Opinions in Pediatrics* 7:13-18 (1995)). The polysaccharide conjugate vaccines have the disadvantage of being costly to produce and manufacture in comparison with many other kinds of vaccines. There is also the  
15 possible risk of problems caused by the cross reactivity between GBS polysaccharides and sialic acid-containing human glycoproteins.

Recent evidence suggests that bacterial surface proteins also may be useful to confer immunity. A protein called Rib which is found on most serotype III strains but rarely  
20 on serotypes Ia, Ib or II confers immunity to challenge with Rib expressing GBS in animal models (Stalhammar-Carlemalm *et al.*, *Journal of Experimental Medicine* 177:1593-1603 (1993)). Another surface protein of interest as a component of a vaccine is the alpha antigen of the C proteins which protected vaccinated mice against lethal infection with strains expressing alpha protein. The amount of this  
25 antigen expressed by GBS strains varies markedly, however an alternative to polysaccharides as antigens is the use of protein antigens derived from GBS. Recent evidence suggest that the GBS surface associated proteins Rib and alpha C protein may be used to confer immunity to GBS infections in experimental model systems

(Stalhammar-Carlemalm *et al.*, (1993) [*supra*], Larsson *et al.*, (1996) [*supra*]). However these two proteins are not conserved in all serotypes of GBS which cause disease in humans. Assuming that these antigens would be immunogenic and elicit protective level responses in humans they would not confer protection against all infections caused by GBS as 10% of infectious Group B streptococci do not express Rib or C protein alpha.

This invention seeks to overcome the problem of vaccination against GBS by using a novel screening method specifically designed to identify those Group B Streptococcus genes encoding bacterial cell surface associated or secreted proteins. The proteins expressed by these genes may be immunogenic, and therefore may be useful in the prevention and treatment of Group B Streptococcus infection. For the purposes of this application, the term immunogenic means that these proteins will elicit a protective immune response within a subject. Using this novel screening method a number of genes encoding novel Group B Streptococcus proteins have been identified.

Thus in a first aspect, the present invention provides a Group B Streptococcus protein, polypeptide or peptide having a sequence selected from those shown in figure 1, or fragments or derivatives thereof.

It will be apparent to the skilled person that proteins and polypeptides included within this group may be cell surface receptors, adhesion molecules, transport proteins, membrane structural proteins, and/or signalling molecules.

Alterations in the amino acid sequence of a protein can occur which do not affect the function of a protein. These include amino acid deletions, insertions and substitutions and can result from alternative splicing and/or the presence of multiple translation

start sites and stop sites. Polymorphisms may arise as a result of the infidelity of the translation process. Thus changes in amino acid sequence may be tolerated which do not affect the protein's function.

5 Thus, the present invention includes derivatives or variants of the proteins, polypeptides, and peptides of the present invention which show at least 50% identity to the proteins, polypeptides and peptides described herein. Preferably the degree of sequence identity is at least 60% and preferably it is above 75%. More preferably still it is above 80%, 90% or even 95%.

10

The term identity can be used to describe the similarity between two polypeptide sequences. A software package well known in the art for carrying out this procedure is the CLUSTAL program. It compares the amino acid sequences of two polypeptides and finds the optimal alignment by inserting spaces in either sequence as appropriate. The amino acid identity or similarity (identity plus conservation of amino acid type) for an optimal alignment can also be calculated using a software package such as BLASTx. This program aligns the largest stretch of similar sequence and assigns a value to the fit. For any one pattern comparison several regions of similarity may be found, each having a different score. One skilled in the art will appreciate that two polypeptides of different lengths may be compared over the entire length of the longer fragment. Alternatively small regions may be compared. Normally sequences of the same length are compared for a useful comparison to be made.

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25 Manipulation of the DNA encoding the protein is a particularly powerful technique for both modifying proteins and for generating large quantities of protein for purification purposes. This may involve the use of PCR techniques to amplify a desired nucleic acid sequence. Thus the sequence data provided herein can be used to

design primers for use in PCR so that a desired sequence can be targeted and then amplified to a high degree.

Typically primers will be at least five nucleotides long and will generally be at least ten  
5 nucleotides long (e.g. fifteen to twenty-five nucleotides long). In some cases primers of at least thirty or at least thirty-five nucleotides in length may be used.

As a further alternative chemical synthesis may be used. This may be automated. Relatively short sequences may be chemically synthesised and ligated together to  
10 provide a longer sequence.

Thus in a further aspect, the present invention provides, a nucleic acid molecule comprising or consisting of a sequence which is:

- 15 (i) any of the DNA sequences set out in figure 1 herein or their RNA equivalents;
- (ii) a sequence which is complementary to any of the sequences of (i);
- (iii) a sequence which codes for the same protein or polypeptide, as those sequences of (i) or (ii);
- (iv) a sequence which shows substantial identity with any of those of (i),  
20 (ii) and (iii); or
- (v) a sequence which codes for a derivative or fragment of a nucleic acid molecule shown in figure 1.

The term identity can also be used to describe the similarity between two individual DNA sequences. The 'bestfit' program (Smith and Waterman, *Advances in applied*  
25 *Mathematics*, 482-489 (1981)) is one example of a type of computer software used to find the best segment of similarity between two nucleic acid sequences, whilst the GAP program enables sequences to be aligned along their whole length and finds the optimal alignment by inserting spaces in either sequence as appropriate.

The present invention includes nucleic acid sequences which show at least 50% identity to the nucleic acid sequences described herein. Preferably the degree of sequence identity is at least 60% and preferably it is above 75%. More preferably still it is above 80%, 90% or even 95%.

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The term 'RNA equivalent' when used above indicates that a given RNA molecule has a sequence which is complementary to that of a given DNA molecule, allowing for the fact that in RNA 'U' replaces 'T' in the genetic code. The nucleic acid molecule may be in isolated, recombinant or chemically synthetic form.

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DNA constructs can readily be generated using methods well known in the art. These techniques are disclosed, for example in J. Sambrook *et al*, *Molecular Cloning 2<sup>nd</sup> Edition*, Cold Spring Harbour Laboratory Press (1989). Modifications of DNA constructs and the proteins expressed such as the addition of promoters, enhancers, signal sequences, leader sequences, translation start and stop signals and DNA stability controlling regions, or the addition of fusion partners may then be facilitated.

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Normally the DNA construct will be inserted into a vector which may be any suitable vector, including plasmid, virus, bacteriophage, transposon, minichromosome, liposome or mechanical carrier. The expression vectors of the invention are DNA constructs suitable for expressing DNA which encodes the desired protein product which may include: (a) a regulatory element (e.g. a promoter, operator, activator, repressor and/or enhancer), (b) a structural or coding sequence which is transcribed into mRNA and (c) appropriate transcription, translation, initiation and termination sequences. The vector may further comprise a selectable marker, for example antibiotic resistance, which facilitates the selection and/or identification of cells containing the vector.

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Expression of the protein is achieved by the transformation or transfection of the vector into a host cell which may be of eukaryotic or prokaryotic origin. For the production of recombinant protein, expression may be inducible expression or  
5 expression only in certain types of cells or both inducible and cell-specific. Particularly preferred among inducible vectors are vectors that can be induced for expression by environmental factors that are easy to manipulate, such as temperature and nutrient additives. A variety of suitable vectors, including constitutive and inducible expression vectors for use in prokaryotic and eukaryotic  
10 hosts, are well known and employed routinely by those skilled in the art.

A great variety of expression vectors can be used to express the Group B Streptococcus protein(s) of the invention. Such vectors include, among others, chromosomal, episomal and virus-derived vectors, for example, vectors derived  
15 from bacterial plasmids, from bacteriophage, from transposons, from yeast elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids, all may be used in accordance with the invention. Generally, any  
20 vector suitable to maintain, propagate or express nucleic acid to express a polypeptide in a host may be used for expression in this regard. Such vectors thus form yet a further aspect of the invention.

The appropriate DNA sequence may be inserted into the vector by any of a variety  
25 of well-known and routine techniques.

The nucleic acid sequence in the expression vector is operatively linked to appropriate expression control sequence(s) including, for instance, a promoter to

direct mRNA transcription. Representatives of such promoters include, but are not limited to, the phage lambda PL promoter, the T3 and T7 promoters, the *E.coli* lac, trp, tac, and  $\lambda$ P<sub>L</sub> promoters, the microbial eukaryote GAL, glucoamylase and cellobiohydrolase promoters and the mammalian metallothionein (mouse) and heat-shock (human) promoters.

In general, expression vectors will contain sites for transcription initiation and termination, and, in the transcribed region, a ribosome binding site for translation. The coding portion of mature transcripts expressed by the constructs will generally include a translation initiating AUG at the beginning and a termination codon appropriately positioned at the end of the polypeptide to be translated.

Representative examples of appropriate hosts for recombinant expression of the Group B Streptococcus protein(s) of the invention include bacterial cells, such as *streptococci*, *staphylococci*, *E.coli*, *streptomyces* and *Bacillus subtilis* cells; fungal cells, such as yeast cells and *Aspergillus* cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS, HeLa and Bowes melanoma cells; and plant cells. Such host cells form yet a further aspect of the present invention.

Microbial cells employed in the expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agent, such methods which are known to those skilled in the art.

The polypeptide can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulphate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose,

chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Well known techniques for refolding protein may be employed to regenerate active conformation when the polypeptide is denatured during isolation and or purification.

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The Group B Streptococcus proteins described herein can additionally be used as target antigens to raise antibodies, or to generate affibodies. These can be used to detect Group B Streptococcus.

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Thus in a further aspect the present invention provides, an antibody, affibody, or a derivative thereof which binds to any one or more of the proteins, polypeptides, peptides, fragments or derivatives thereof, as described herein.

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Antibodies within the scope of the present invention may be monoclonal or polyclonal. Polyclonal antibodies can be raised by stimulating their production in a suitable animal host (e.g. a mouse, rat, guinea pig, rabbit, sheep, goat or monkey) when a protein as described herein, or a homologue, derivative or fragment thereof, is injected into the animal. If desired, an adjuvant may be administered together with the protein. Well-known adjuvants include Freund's adjuvant (complete and incomplete) and aluminium hydroxide. The antibodies can then be purified by virtue of their binding to a protein as described herein and by many other means well-known to those skilled in the art.

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Monoclonal antibodies can be produced from hybridomas. These can be formed by fusing myeloma cells and spleen cells which produce the desired antibody in order to form an immortal cell line. Thus the well-known Kohler & Milstein technique (*Nature* 256 (1975)) or subsequent variations upon this technique can be used.

Techniques for producing monoclonal and polyclonal antibodies that bind to a particular polypeptide/protein are now well developed in the art. They are discussed in standard immunology textbooks, for example in Roitt *et al*, *Immunology* second edition (1989), Churchill Livingstone, London.

5

In addition to whole antibodies, the present invention includes derivatives thereof which are capable of binding to proteins etc as described herein. Thus the present invention includes antibody fragments and synthetic constructs. Examples of antibody fragments and synthetic constructs are given by Dougall *et al* ., *Tibtech* **12** 372-379 (September 1994).

10

Antibody fragments include, for example, Fab, F(ab')<sub>2</sub> and Fv fragments. Fv fragments can be modified to produce a synthetic construct known as a single chain Fv (scFv) molecule. This includes a peptide linker covalently joining V<sub>h</sub> and V<sub>l</sub> regions, which contributes to the stability of the molecule. Other synthetic constructs that can be used include CDR peptides. These are synthetic peptides comprising antigen-binding determinants. Peptide mimetics may also be used. These molecules are usually conformationally restricted organic rings that mimic the structure of a CDR loop and that include antigen-interactive side chains.

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Synthetic constructs include chimaeric molecules. Thus, for example, humanised (or primatised) antibodies or derivatives thereof are within the scope of the present invention. An example of a humanised antibody is an antibody having human framework regions, but rodent hypervariable regions. Ways of producing chimaeric antibodies are discussed for example by Morrison *et al* in *PNAS*, **81**, 6851-6855 (1984) and by Takeda *et al* in *Nature*. **314**, 452-454 (1985).

25

Synthetic constructs also include molecules comprising an additional moiety that provides the molecule with some desirable property in addition to antigen binding. For example the moiety may be a label (e.g. a fluorescent or radioactive label). Alternatively, it may be a pharmaceutically active agent.

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Affibodies are proteins which are found to bind to target proteins with a low dissociation constant. They are selected from phage display libraries expressing a segment of the target protein of interest (Nord K, Gunneriusson E, Ringdahl J, Stahl S, Uhlen M, Nygren PA, Department of Biochemistry and Biotechnology, Royal Institute of Technology (KTH), Stockholm, Sweden).

10

In a further aspect the invention provides an immunogenic composition comprising one or more proteins, polypeptides, peptides, fragments or derivatives thereof, or nucleotide sequences described herein. The immunogenic composition may include nucleic acid sequences ID-65 and/or ID-66 as described herein. Alternatively, the immunogenic composition may comprise proteins/polypeptides including ID-65, ID-83, ID-89, ID-93 and/or ID-96 as described herein, or fragments or derivatives thereof. A composition of this sort may be useful in the treatment or prevention of Group B Streptococcus infection in subject. In a preferred aspect of the invention the immunogenic composition is a vaccine.

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In other aspects the invention provides:

- i) Use of an immunogenic composition as described herein in the preparation of a medicament for the treatment or prophylaxis of Group B Streptococcus infection. Preferably the medicament is a vaccine.

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- ii) A method of detection of Group B Streptococcus which comprises the step of bringing into contact a sample to be tested with at least one antibody, affibody, or a derivative thereof, as described herein.
- 5    iii) A method of detection of Group B Streptococcus which comprises the step of bringing into contact a sample to be tested with at least one protein, polypeptide, peptide, fragments or derivatives as described herein.
- 10    iv) A method of detection of Group B Streptococcus which comprises the step of bringing into contact a sample to be tested with at least one nucleic acid molecule as described herein.
- 15    v) A kit for the detection of Group B Streptococcus comprising at least one antibody, affibody, or derivatives thereof, described herein.
- 15    vi) A kit for the detection of Group B Streptococcus comprising at least one Group B Streptococcus protein, polypeptide, peptide, fragment or derivative thereof, as described herein.
- 20    vii) A kit for the detection of Group B Streptococcus comprising at least one nucleic acid of the invention.

As described previously, the novel proteins described herein are identified and isolated using a screening method which specifically identifies those Group B  
25    Streptococcus genes encoding bacterial cell envelope associated or secreted proteins.

Given that the inventors have identified a group of important proteins, such proteins are potential targets for anti-microbial therapy. It is necessary, however, to

determine whether each individual protein is essential for the organism's viability. Thus, the present invention also provides a method of determining whether a protein or polypeptide as described herein represents a potential anti-microbial target which comprises inactivating said protein and determining whether Group B Streptococcus is still viable.

A suitable method for inactivating the protein is to effect selected gene knockouts, ie prevent expression of the protein and determine whether this results in a lethal change. Suitable methods for carrying out such gene knockouts are described in Li *et al* , *P.N.A.S.*, **94**:13251-13256 (1997) and Kolkman *et al.*, *Journal of Biological Chemistry* **272**: 19502-19508 (1997); Kolkman *et al.*, *Journal of Bacteriology* **178**: 3736-3741 (1996).

In a final aspect the present invention provides the use of an agent capable of antagonising, inhibiting or otherwise interfering with the function or expression of a protein or polypeptide of the invention in the manufacture of a medicament for use in the treatment or prophylaxis of Group B Streptococcus infection.

The invention will now be described by means of the following examples which should not in any way be construed as limiting. The examples refer to the figures in which:

Fig 1: (A) Shows a number of full length nucleotide sequences encoding antigenic Group B Streptococcus proteins and the corresponding amino acid sequences.

Fig 2: Shows the results of vaccine trials using the proteins ID-65 and ID-66;

Fig 3: Shows a number of oligonucleotide primers used in the screening process

**nucS1** primer designed to amplify a mature form of the *nuc* A gene

**nucS2-** primer designed to amplify a mature form of the *nuc* A gene.

5 **nucS3** primer designed to amplify a mature form of the *nuc* A gene

**nucR** primer designed to amplify a mature form of the *nuc* A gene

**nucseq** primer designed to sequence DNA cloned into the pTREP-Nuc vector  
**pTREPF** nucleic acid sequence containing recognition site for *ECORV*. Used  
 for cloning fragments into pTREX7.

10 **pTREPR** nucleic acid sequence containing recognition site for *BAMH1*.  
 Used for cloning fragments into pTREX7.

**PUCF** forward sequencing primer, enables direct sequencing of cloned DNA  
 fragments.

15 **VR** example of gene specific primer used to obtain further antigen DNA  
 sequence by the method of DNA walking.

**V1** example of gene specific primer used to obtain further antigen DNA  
 sequence by the method of DNA walking.

**V2** example of gene specific primer used to obtain further antigen DNA  
 sequence by the method of DNA walking.

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Fig 4: (i) Schematic presentation of the nucleotide sequence of the unique  
 gene cloning site immediately upstream of the mature *nuc* gene in pTREP1-  
*nuc1*, pTREP1-*nuc2* and pTREP1-*nuc3*. Each of the pTREP-*nuc* vectors  
 contain an *EcoRV* (a *SmaI* site in pTREP1-*nuc2*) cleavage site which allows  
 25 cloning of genomic DNA fragments in 3 different frames with respect to the  
 mature *nuc* gene.

(ii) A physical and genetic summary map of the pTREP1-*nuc* vectors. The  
 expression cassette incorporating *nuc*, the macrolides, lincosamides and

streptogramin B (MLS) resistance determinant, and the replicon (rep) *Ori-pAM $\beta$ 1* are depicted (not drawn to scale).

(iii) Schematic presentation of the expression cassette showing the various sequence elements involved in gene expression and location of unique restriction endonuclease sites (not drawn to scale).

Fig 5: SDS-PAGE analysis of a purified preparation of the His-tagged ID-65 and ID-83 protein antigens (predicted molecular weights of 57,144 and 25,000 daltons respectively) on a 12% polyacrylamide gel. Lanes: MW, molecular weight standards; 1, His-tagged ID-65 protein; 2, His-tagged ID-83 protein

Fig 6: SDS PAGE analysis of a purified preparation of the His-tagged ID-93 protein antigen (predicted molecular weight = 28,000 daltons) on a 12% polyacrylamide gel.

Lanes: MW, molecular weight standards; 1, His-tagged ID-93 protein.

Fig 7: SDS PAGE analysis of a purified preparation of the His-tagged ID-89 and ID-96 protein antigens (predicted molecular weights of 35,000 and 31,000 daltons respectively) on a 12% polyacrylamide gel.

Lanes: MW, molecular weight standards; 1, His-tagged ID-89 protein; 2, His-tagged ID-96 protein.

Fig 8: IgG Titres against the ID-65 and ID-83 proteins

1 = ID-65 + Alum Group – Bleed at 5 weeks

2 = PBS + Alum Control Group – Bleed at 5 weeks

(For groups 1 and 2, ELISAs were performed on purified ID-65 protein)

3 = ID-83 + Alum Group – Bleed at 5 weeks

17.

4 = PBS+ Alum Control Group – Bleed at 5 weeks

(For groups 3 and 4, ELISAs were performed on purified ID-83 protein)

Fig 9: Shows the results of vaccine trials using the protein ID-93.

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Fig 10: IgG titres against the ID-93 protein.

1 = ID-93+ Alum Group – Bleed at 3 weeks

2 = ID-93+ Alum Group – Bleed at 6 weeks

3 = PBS+ Alum Control Group – Bleed at 3 weeks

10 4 = PBS+ Alum Control Group – Bleed at 6 weeks

Fig 11: IgG titres against the ID-89 and ID-96 proteins

1 = ID-89+ TitreMax Gold Group – Bleed at 3 weeks

2 = ID-89+ TitreMax Gold – Bleed at 6 weeks

15 3 = PBS+ TitreMax Gold Control Group – Bleed at 3 weeks

4 = PBS+ TitreMax Gold Control Group – Bleed at 6 weeks

5 = ID-96+ TitreMax Gold Group – Bleed at 3 weeks

6 = ID-96+ TitreMax Gold Group – Bleed at 6 weeks

7 = PBS+ TitreMax Gold Control Group – Bleed at 3 weeks

20 8 = PBS+ TitreMax Gold Control Group – Bleed at 6 weeks

For Groups 1-4, ELISAs were performed on purified ID-89 protein.

For Groups 5-6, ELISAs were performed on purified ID-96 protein.

Fig 12: Southern blot analysis of genomic DNA. Genomic DNA from each of the strains listed in Table 7 was digested completely with *Hin* DIII (NEB) and electrophoresed at 40 Volts for 6 hours in 0.8% agarose, transferred onto Hybond N<sup>+</sup> (Amersham) membrane by Southern blot and hybridised with the

25

digoxigenin-labelled *rib* gene probe. Specifically bound DNA probe was identified using the DIG Nucleic Acid Detection Kit (Boehringer Mannheim).

5 Fig 13: Southern blot analysis of genomic DNA. Genomic DNA from each of the strains listed in Table 6 was digested completely with *Hin* DIII (NEB) and electrophoresed at 40 Volts for 6 hours in 0.8% agarose, transferred onto Hybond N<sup>+</sup> (Amersham) membrane by Southern blot and hybridised with the digoxigenin-labelled ID-65 gene probe. Specifically bound DNA probe was identified using the DIG Nucleic Acid Detection Kit (Boehringer Mannheim).

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Fig 14: Southern blot analysis of genomic DNA. Genomic DNA from each of the strains listed in Table 6 was digested completely with *Hin* DIII (NEB) and electrophoresed at 40 Volts for 6 hours in 0.8% agarose, transferred onto Hybond N<sup>+</sup> (Amersham) membrane by Southern blot and hybridised with the digoxigenin-labelled ID-89 gene probe. Specifically bound DNA probe was identified using the DIG Nucleic Acid Detection Kit (Boehringer Mannheim).

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Fig 15: Southern blot analysis of genomic DNA. Genomic DNA from each of the strains listed in Table 6 was digested completely with *Hin* DIII (NEB) and electrophoresed at 40 Volts for 6 hours in 0.8% agarose, transferred onto Hybond N<sup>+</sup> (Amersham) membrane by Southern blot and hybridised with the digoxigenin-labelled ID-93 gene probe. Specifically bound DNA probe was identified using the DIG Nucleic Acid Detection Kit (Boehringer Mannheim).

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25 Fig 16: Southern blot analysis of genomic DNA. Genomic DNA from each of the strains listed in Table 6 was digested completely with *Eco* RI (NEB) and electrophoresed at 40 Volts for 6 hours in 0.8% agarose, transferred onto Hybond N<sup>+</sup> (Amersham) membrane by Southern blot and hybridised with the

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digoxigenin-labelled ID-96 gene probe. Specifically bound DNA probe was identified using the DIG Nucleic Acid Detection Kit (Boehringer Mannheim).

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### Example 1

Gene/partial gene sequences putatively encoding exported proteins in *S. agalactiae* have been identified, unless stated otherwise, using the nuclease screening system described herein *vis*, the LEEP (Lactococcus Expression of Exported Proteins) system. These have been further analysed to remove artefacts. The nucleotide sequences of genes identified using the screening system have been characterised using a number of parameters described below.

1. All putative surface proteins are analysed for leader/signal peptide sequences. Bacterial signal peptide sequences share a common design. They are characterised by a short positively charged N-terminus (N region) immediately preceding a stretch of hydrophobic residues (central portion-h region) followed by a more polar C-terminal portion which contains the cleavage site (c-region). Computer software is used to perform hydropathy profiling of putative proteins (Marcks, *Nuc. Acid. Res.*, **16**:1829-1836 (1988)) which is used to identify the distinctive hydrophobic portion (h-region) typical of leader peptide sequences. In addition, the presence/absence of a potential ribosomal binding site (Shine-Dalgarno sequence required for translation) is also noted.

2. All putative surface protein sequences are used to search the OWL sequence database which includes a translation of the GENBANK and SWISSPROT database.. This allows identification of similar sequences which may have been previously characterised not only at the sequence level but at a functional level. It

may also provide information indicating that these proteins are indeed surface related and not artefacts.

3. Putative *S. agalactiae* surface proteins are also assessed for their novelty. Some of the identified proteins may or may not possess a typical leader peptide sequence and may not show homology with any DNA/protein sequences in the database. Indeed these proteins may indicate the primary advantage of our screening method, i.e. isolating atypical surface-related proteins, which would have been missed in all previously described screening protocols.

10 The construction of three reporter vectors and their use in *L. lactis* to identify and isolate genomic DNA fragments from pathogenic bacteria encoding secreted or surface associated proteins is now described.

#### Construction of the pTREP1-*nuc* series of reporter vectors

##### 15 (a) Construction of expression plasmid pTREP1

The pTREP1 plasmid is a high-copy number (40-80 per cell) theta-replicating gram positive plasmid, which is a derivative of the pTREX plasmid which is itself a derivative of the previously published pIL253 plasmid. pIL253 incorporates the broad Gram-positive host range replicon of pAM $\beta$ 1 (Simon and Chopin, *Biochemie* 20 **70**: 559-566 (1988)) *L. lactis* sex-factor. pIL253 also lacks the *tra* function which is necessary for transfer or efficient mobilisation by conjugative parent plasmids exemplified by pIL501. The Enterococcal pAM $\beta$ 1 replicon has previously been transferred to various species including *Streptococcus*, *Lactobacillus* and *Bacillus* 25 species as well as *Clostridium acetobutylicum*, (LeBlanc *et al.*, *Proceedings of the National Academy of Science USA* **75**:3484-3487 (1978)) indicating the potential broad host range utility. The pTREP1 plasmid represents a constitutive transcription vector.

The pTREX vector was constructed as follows. An artificial DNA fragment containing a putative RNA stabilising sequence, a translation initiation region (TIR), a multiple cloning site for insertion of the target genes and a transcription terminator was created by annealing 2 complementary oligonucleotides and extending with Tfl DNA polymerase. The sense and anti-sense oligonucleotides contained the recognition sites for NheI and BamHI at their 5' ends respectively to facilitate cloning. This fragment was cloned between the XbaI and BamHI sites in pUC19NT7, a derivative of pUC19 which contains the T7 expression cassette from pLET1 (Wells *et al.*, *J. Appl. Bacteriol.* **74**:629-636 (1993)) cloned between the EcoRI and HindIII sites. The resulting construct was designated pUCLEX. The complete expression cassette of pUCLEX was then removed by cutting with HindIII and blunting followed by cutting with EcoRI before cloning into EcoRI and SacI (blunted) sites of pIL253 to generate the vector pTREX (Wells and Schofield, *In Current advances in metabolism, genetics and applications-NATO ASI Series. H* **98**:37-62. (1996)). The putative RNA stabilising sequence and TIR are derived from the *Escherichia coli* T7 bacteriophage sequence and modified at one nucleotide position to enhance the complementarity of the Shine Dalgarno (SD) motif to the ribosomal 16s RNA of *Lactococcus lactis* (Schofield *et al.* pers. coms. University of Cambridge Dept. Pathology.).

A *Lactococcus lactis* MG1363 chromosomal DNA fragment exhibiting promoter activity which was subsequently designated P7 was cloned between the EcoRI and BglII sites present in the expression cassette, creating pTREX7. This active promoter region had been previously isolated using the promoter probe vector pSB292 (Waterfield *et al.*, *Gene* **165**:9-15 (1995)). The promoter fragment was amplified by PCR using the Vent DNA polymerase according to the manufacturer.

The pTREP1 vector was then constructed as follows. An artificial DNA fragment which included a transcription terminator, the forward pUC sequencing primer, a promoter multiple cloning site region and a universal translation stop sequence was created by annealing two overlapping partially complementary synthetic oligonucleotides together and extending with sequenase according to manufacturers instructions. The sense and anti-sense (pTREP<sub>F</sub> and pTREP<sub>R</sub>) oligonucleotides contained the recognition sites for EcoRV and BamHI at their 5' ends respectively to facilitate cloning into pTREX7. The transcription terminator was that of the *Bacillus penicillinase* gene, which has been shown to be effective in *Lactococcus* (Jos *et al.*, *Applied and Environmental Microbiology* **50**:540-542 (1985)). This was considered necessary as expression of target genes in the pTREX vectors was observed to be leaky and is thought to be the result of cryptic promoter activity in the origin region (Schofield *et al.* pers. coms. University of Cambridge Dept. Pathology.). The forward pUC primer sequencing was included to enable direct sequencing of cloned DNA fragments. The translation stop sequence which encodes a stop codon in 3 different frames was included to prevent translational fusions between vector genes and cloned DNA fragments. The pTREX7 vector was first digested with EcoRI and blunted using the 5' - 3' polymerase activity of T4 DNA polymerase (NEB) according to manufacturer's instructions. The EcoRI digested and blunt ended pTREX7 vector was then digested with Bgl II thus removing the P7 promoter. The artificial DNA fragment derived from the annealed synthetic oligonucleotides was then digested with EcoRV and Bam HI and cloned into the EcoRI(blunted)-Bgl II digested pTREX7 vector to generate pTREP. A *Lactococcus lactis* MG1363 chromosomal promoter designated P1 was then cloned between the EcoRI and BglII sites present in the pTREP expression cassette forming pTREP1. This promoter was also isolated using the promoter probe vector pSB292 and characterised by Waterfield *et al.*, (1995) [*supra*]. The P1 promoter fragment was originally amplified by PCR using vent DNA polymerase according to manufacturers

instructions and cloned into the pTREX as an EcoRI-BglIII DNA fragment. The EcoRI-BglIII P1 promoter containing fragment was removed from pTREX1 by restriction enzyme digestion and used for cloning into pTREP (Schofield *et al.* pers. coms. University of Cambridge, Dept. Pathology.).

5

**(b) PCR amplification of the *S. aureus* nuc gene.**

The nucleotide sequence of the *S. aureus* nuc gene (EMBL database accession number V01281) was used to design synthetic oligonucleotide primers for PCR  
10 amplification. The primers were designed to amplify the mature form of the nuc gene designated nucA which is generated by proteolytic cleavage of the N-terminal 19 to 21 amino acids of the secreted propeptide designated Snase B (Shortle, 1983 [supra]). Three sense primers (nucS1, nucS2 and nucS3, shown in figure 3) were designed, each one having a blunt-ended restriction endonuclease cleavage site for  
15 EcoRV or SmaI in a different reading frame with respect to the nuc gene. Additionally BglIII and BamHI were incorporated at the 5' ends of the sense and anti-sense primers respectively to facilitate cloning into BamHI and BglIII cut pTREP1. The sequences of all the primers are given in figure 3. Three nuc gene DNA fragments encoding the mature form of the nuclease gene (NucA) were amplified by  
20 PCR using each of the sense primers combined with the anti-sense primer. The nuc gene fragments were amplified by PCR using *S. aureus* genomic DNA template, Vent DNA Polymerase (NEB) and the conditions recommended by the manufacturer. An initial denaturation step at 93°C for 2 min was followed by 30 cycles of denaturation at 93°C for 45 sec, annealing at 50°C for 45 seconds, and extension at  
25 73°C for 1 minute and then a final 5 min extension step at 73°C. The PCR amplified products were purified using a Wizard clean up column (Promega) to remove unincorporated nucleotides and primers.

**(c) Construction of the pTREP1-*nuc* vectors**

The purified *nuc* gene fragments described in section b were digested with Bgl II and BamHI using standard conditions and ligated to BamHI and BglII cut and dephosphorylated pTREP1 to generate the pTREP1-*nuc*1, pTREP1-*nuc*2 and pTREP1-*nuc*3 series of reporter vectors. These vectors are described in figure 4. General molecular biology techniques were carried out using the reagents and buffers supplied by the manufacturer or using standard techniques (Sambrook and Maniatis, Molecular cloning: A laboratory manual. Cold Spring Harbor Laboratory Press: Cold Spring Harbour (1989)). In each of the pTREP1-*nuc* vectors the expression cassette comprises a transcription terminator, lactococcal promoter P1, unique cloning sites (Bgl II, EcoRV or SmaI) followed by the mature form of the *nuc* gene and a second transcription terminator. Note that the sequences required for translation and secretion of the *nuc* gene were deliberately excluded in this construction. Such elements can only be provided by appropriately digested foreign DNA fragments (representing the target bacterium) which can be cloned into the unique restriction sites present immediately upstream of the *nuc* gene.

**(d) Screening for secreted proteins in Group B Streptococcus.**

Genomic DNA isolated from Group B Streptococcus (*S. agalactiae*) was digested with the restriction enzyme Tru9I. This enzyme which recognises the sequence 5'-TTAA -3' was used because it cuts A/T rich genomes efficiently and can generate random genomic DNA fragments within the preferred size range (usually averaging 0.5 - 1.0 kb). This size range was preferred because there is an increased probability that the P1 promoter can be utilised to transcribe a novel gene sequence. However, the P1 promoter may not be necessary in all cases as it is possible that many Streptococcal promoters are recognised in *L. lactis*. DNA fragments of different size ranges were purified from partial Tru9I digests of *S. agalactiae* genomic DNA. As

the Tru 9I restriction enzyme generates staggered ends the DNA fragments had to be made blunt ended before ligation to the EcoRV or SmaI cut pTREP1-*nuc* vectors. This was achieved by the partial fill-in enzyme reaction using the 5'-3' polymerase activity of Klenow enzyme. Briefly Tru9I digested DNA was dissolved in a solution (usually between 10-20  $\mu$ l in total) supplemented with T4 DNA ligase buffer (New England Biolabs; NEB) (1X) and 33  $\mu$ M of each of the required dNTPs, in this case dATP and dTTP. Klenow enzyme was added (1 unit Klenow enzyme (NEB) per  $\mu$ g of DNA) and the reaction incubated at 25°C for 15 minutes. The reaction was stopped by incubating the mix at 75°C for 20 minutes. EcoRV or SmaI digested pTREP-*nuc* plasmid DNA was then added (usually between 200-400 ng). The mix was then supplemented with 400 units of T4 DNA ligase (NEB) and T4 DNA ligase buffer (1X) and incubated overnight at 16°C. The ligation mix was precipitated directly in 100% Ethanol and 1/10 volume of 3M sodium acetate (pH 5.2) and used to transform *L. lactis* MG1363 (Gasson, *J. Bacteriol.* **154**:1-9 (1983)). Alternatively, the gene cloning site of the pTREP-*nuc* vectors also contains a BglII site which can be used to clone for example Sau3AI digested genomic DNA fragments.

*L. lactis* transformant colonies were grown on brain heart infusion agar and nuclease secreting (*Nuc*<sup>+</sup>) clones were detected by a toluidine blue-DNA-agar overlay (0.05 M Tris pH 9.0, 10 g of agar per litre, 10 g of NaCl per liter, 0.1 mM CaCl<sub>2</sub>, 0.03 % wt/vol. salmon sperm DNA and 90 mg of Toluidine blue O dye) essentially as described by Shortle, 1983 [*supra*], and Le Loir *et al.*, 1994 [*supra*]). The plates were then incubated at 37°C for up to 2 hours. Nuclease secreting clones develop an easily identifiable pink halo. Plasmid DNA was isolated from *Nuc*<sup>+</sup> recombinant *L. lactis* clones and DNA inserts were sequenced on one strand using the NucSeq sequencing primer described in figure 3, which sequences directly through the DNA insert.

## Example 2

### Preparation of a *S. agalactiae* standard inoculum

#### 5 Strain validation

*S. agalactiae* serotype III (strain 97/0099) is a recent clinical isolate derived from the cerebral spinal fluid of a new born baby suffering from meningitis. This haemolytic strain of Group B Streptococcus was epidemiologically tested and validated at the Respiratory and Systemic Infection Laboratory, PHLS Central Public Health Laboratory, 61 Colindale Avenue, London NW9 5HT. The strain was subcultured only twice prior to its arrival in the laboratory. Upon its arrival on an agar slope, a sweep of 4-5 colonies was immediately used to inoculate a Todd Hewitt/5% horse blood broth which was incubated overnight statically at 37°C. 0.5 ml aliquots of this overnight culture were then used to make 20% glycerol stocks of the bacterium for long-term storage at -70°C. Glycerol stocks were streaked on Todd Hewitt/5% horse blood agar plates to confirm viability.

#### *In vivo* passaging of Group B Streptococcus

A frozen culture (described under strain validation) of *S. agalactiae* serotype III (strain 97/0099) was streaked to single colonies on Todd-Hewitt/5% blood agar plates, which were incubated overnight at 37°C. A sweep of 4-5 colonies was used to inoculate a Todd Hewitt/5% horse blood broth, which was again incubated overnight. A 0.5 ml aliquot from this overnight culture was used to inoculate a 50 ml Todd Hewitt broth (1:100 dilution) which was incubated at 37°C. 10-fold serial dilutions of the overnight culture were made (since virulence of this strain was unknown) and each was passaged intra-peritoneally (IP) in CBA/ca mice in duplicate. Viable counts were performed on the various inocula used in the passage. Groups of mice were challenged with various concentrations of the pathogen ranging from  $10^8$  to  $10^4$  colony forming units (cfu). Mice that developed symptoms were terminally anaesthetized and cardiac punctures were performed (Only mice that had

been challenged with the highest doses, i.e.  $1 \times 10^8$  cfu, developed symptoms). The retrieved unclotted blood was used to inoculate directly a 50ml serum broth (Todd Hewitt/20% inactivated foetal calf serum). The culture was constantly monitored and allowed to grow to late logarithmic phase. The presence of blood in the medium interfered with OD<sub>600nm</sub> readings as it was being increasingly lysed with increasing growth of the bacterium, hence the requirement to constantly monitor the culture. Upon reaching late logarithmic phase/early stationary phase, the culture was transferred to a fresh 50 ml tube in order to exclude dead bacterial cells and remaining blood cells which would have sedimented at the bottom of the tube. 0.5 ml aliquots were then transferred to sterile cryovials, frozen in liquid nitrogen and stored at  $-70^{\circ}\text{C}$ . A viable count was carried out on a single standard inoculum aliquot in order to determine bacterial numbers. This was determined to be approximately  $5 \times 10^8$  cfu per ml.

#### **15 Intra-peritoneal Challenge and virulence testing of Group B Streptococcus standard inoculum**

To determine if the standard inoculum was suitably virulent for use in a vaccine trial, challenges were carried out using a dose range. Frozen standard inoculum strain aliquots were allowed to thaw at room temperature. From viable count data the number of cfu per ml was already known for the standard inoculum. Initially, serial dilutions of the standard inoculum were made in Todd Hewitt broth and mice were challenged intra-peritoneally with doses ranging from  $1 \times 10^8$  to  $1 \times 10^4$  cfu in a 500  $\mu\text{l}$  volume of Todd Hewitt broth. The survival times of mouse groups injected with different doses of the bacterium were compared. The standard inoculum was determined to be suitably virulent and a dose of  $1 \times 10^6$  cfu was considered close to optimal for further use in vaccine trials. Further optimisation was carried out by comparing mice challenged with doses ranging between  $5 \times 10^5$  and  $5 \times 10^6$  cfu. The optimal dose was estimated to be approximately  $2.5 \times 10^6$  cfu. This represented

a 100% lethal dose and was repeatedly consistent with end-points as determined by survival times being clustered within a narrow time-range. Throughout all these experiments, challenged mice were constantly monitored to clarify symptoms, stages of symptom development as well as calculating survival times.

5

**Screening Group B Streptococcal LEEP derived genes in DNA vaccination experiments.**

**pcDNA3.1+ as a DNA vaccine vector**

10 The commercially available pcDNA3.1+ plasmid (Invitrogen), referred to as pcDNA3.1 henceforth, was used as a vector in all DNA immunisation experiments involving gene targets derived using the LEEP system unless stated otherwise. pcDNA 3.1 is designed for high-level stable and transient expression in mammalian cells and has been used widely and successfully as a host vector to test candidate  
15 genes from a variety of pathogens in DNA vaccination experiments (Zhang *et al.*, *Infection and Immunity* **176**: 1035-40 (1997); Kurar and Splitter, *Vaccine* **15**: 1851-57 (1997); Anderson *et al.*, *Infection and Immunity* **64**: 3168-3173 (1996)).

The vector possesses a multiple cloning site which facilitates the cloning of multiple  
20 gene targets downstream of the human cytomegalovirus (CMV) immediate-early promoter/enhancer which permits efficient, high-level expression of the target gene in a wide variety of mammalian cells and cell types including both muscle and immune cells. This is important for optimal immune response as it remains unknown as to which cells types are most important in generating a protective response *in*  
25 *vivo*. The plasmid also contains the ColE1 origin of replication which allows convenient high-copy number replication and growth in *E. coli* and the ampicillin resistance gene (B- lactamase) for selection in *E. coli*. In addition pcDNA 3.1

possesses a T7 promoter/priming site upstream of the MCS which allows for *in vitro* transcription of a cloned gene in the sense orientation.

#### **Preparation of DNA vaccines**

5 Oligonucleotide primers were designed for each individual gene of interest derived using the LEEP system unless stated otherwise. Each gene was examined thoroughly, and where possible, primers were designed such that they targeted that portion of the gene believed to encode only the mature portion of the protein (APPENDIX I); the intention being to express those sequences that encode only the  
10 mature portion of a target gene protein to would facilitate its correct folding when expressed in mammalian cells. For example, in the majority of cases primers were designed such that putative N-terminal signal peptide sequences would not be included in the final amplification product to be cloned into the pcDNA3.1 expression vector. The signal peptide directs the polypeptide precursor to the cell  
15 membrane via the protein export pathway where it is normally cleaved off by signal peptidase I (or signal peptidase II if a lipoprotein). Hence the signal peptide does not make up any part of the mature protein whether it be displayed on the bacterium's surface or secreted. Where an N-terminal leader peptide sequence was not immediately obvious, primers were designed to target the whole of the gene  
20 sequence for cloning and ultimately, expression in pcDNA3.1.

All forward and reverse oligonucleotide primers incorporated appropriate restriction enzyme sites to facilitate cloning into the pcDNA3.1 MCS region. All forward primers were also designed to include the conserved Kozak nucleotide sequence 5'-  
25 gccacc-3' immediately upstream of an 'atg' translation initiation codon in frame with the target gene insert. The Kozak sequence facilitates the recognition of initiator sequences by eukaryotic ribosomes. Typically, a forward primer incorporating a BamH1 restriction enzyme site the primer would begin with the sequence 5'-

cgggatccgccaccatg-3', followed by a sequence homologous to the 5' end of that part of a gene being amplified. All reverse primers incorporated a Not I restriction enzyme site sequence 5' -ttcgggccgc-3'. All gene-specific forward and reverse primers were designed with compatible melting temperatures to facilitate their amplification.

All gene targets were amplified by PCR from *S. agalactiae* genomic DNA template using Vent DNA polymerase (NEB) or rTth DNA polymerase (PE Applied Biosystems) using conditions recommended by the manufacturer. A typical amplification reaction involved an initial denaturation step at 95°C for 2 minutes followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at the appropriate melting temperature for 30 seconds, and extension at 72°C for 1 minute (1 minute per kilobase of DNA being amplified). This was followed by a final extension period at 72°C for 10 minutes. All PCR amplified products were extracted once with phenol chloroform (2:1:1) and once with chloroform (1:1) and ethanol precipitated. Specific DNA fragments were isolated from agarose gels using the QIAquick Gel Extraction Kit (Qiagen). The purified amplification gene DNA fragments were digested with the appropriate restriction enzymes and cloned into the pcDNA3.1 plasmid vector using *E. coli* as a host. Successful cloning and maintenance of genes was confirmed by restriction mapping and by DNA sequencing. Recombinant plasmid DNA was isolated on a large scale (> 1.5 mg) using Plasmid Mega Kits (Qiagen).

#### **DNA vaccination trials**

DNA vaccine trials in mice were accomplished by the administration of DNA to 6 week old CBA/ca mice (Harlan, UK). Mice to be vaccinated were divided into groups of six and each group was immunised with recombinant pcDNA3.1 plasmid DNA containing a specific target-gene sequence derived using the LEEP system unless stated otherwise. A total of 100 µg of DNA in Dulbecco's PBS (Sigma) was

injected intramuscularly into the tibialis anterior muscle of both hind legs. Four weeks later this procedure was repeated using the same amount of DNA. For comparison, control mice groups were included in all vaccine trials. These control groups were either not DNA-vaccinated or were immunised with non-recombinant pcDNA3.1 plasmid DNA only, using the same time course described above. Four weeks after the second immunisation, all mice groups were challenged intra-peritoneally with a lethal dose of *S. agalactiae* serotype III (strain 97/0099). The actual number of bacteria administered was determined by plating serial dilutions of the inoculum on Todd-Hewitt/5% blood agar plates. All mice were killed 3 or 4 days after infection. During the infection process, challenged mice were monitored for the development of symptoms associated with the onset of *S. agalactiae* induced-disease. Typical symptoms in an appropriate order included piloerection, an increasingly hunched posture, discharge from eyes, increased lethargy and reluctance to move which was often the result of apparent paralysis in the lower body/hind leg region. The latter symptoms usually coincided with the development of a moribund state at which stage the mice were culled to prevent further suffering. These mice were deemed to be very close to death, and the time of culling was used to determine a survival time for statistical analysis. Where mice were found dead, a survival time was calculated by averaging the time when a particular mouse was last observed alive and the time when found dead, in order to determine a more accurate time of death. The results of this trial are shown in Table 1 and presented graphically in Figure 2.

### Interpretation of Results

A positive result was taken as any DNA sequence that was cloned and used in challenge experiments as described above and gave protection against that challenge. DNA sequences were determined to be protective;

-if that DNA sequence gave statistically significant protection to mice as compared to control mice (to a 95% confidence level ( $p > 0.05$ ) as determined using the Mann-Whitney U test .

-if that DNA sequence was marginal or non-significant using Mann-Whitney but showed some protective features. For example, one or more outlying mice may survive for significantly longer time periods when compared with control mice. Alternatively, the time to first death may also be prolonged when compared to counterpart mice in control groups. It is acceptable to allow marginal or non-significant results to be considered as potential positives when it is possible that the clarity of some results may be affected by problems associated with the administration of the DNA vaccine. Indeed, much varied survival times may reflect different levels of immune response between different members of a given group.

Table 1

**LEEP DNA immunisation and GBS challenge Experiment**

**Statistical analysis of survival times**

|                | Mean Survival Times (hours) |               |               |
|----------------|-----------------------------|---------------|---------------|
|                | UnVacc                      | 3-60(ID-65)   | 3-5(ID-66)    |
| 1              | 27.583                      | 54.416        | 42.916        |
| 2              | 27.583                      | 31.000        | 42.916        |
| 3              | 24.583                      | 43.000        | 32.874        |
| 4              | 22.250                      | 34.916        | 42.916        |
| 5              | 35.916                      | 38.958        | 27.333        |
| 6              | 22.250                      | 34.916        | 30.916        |
| <b>Mean</b>    | <b>27.583</b>               | <b>40.458</b> | <b>37.791</b> |
| <b>sd</b>      | <b>5.1691</b>               | <b>8.9959</b> | <b>7.2860</b> |
| <b>p value</b> |                             | <b>0.0098</b> | <b>0.0215</b> |

**p value** refers to statistical significance when compared to unvaccinated controls.

**Comment****ID-65 (3-60)**

5

Mice immunised with the '3-60 (ID-65)' DNA vaccine exhibited significantly longer survival times when compared with the unvaccinated control group.

**ID-66 (3-5)**

10

Mice immunised with the '3-5 (ID-66)' DNA vaccine exhibited significantly longer survival times when compared with the unvaccinated control group.

**Example 3**

15

**Expression and Screening Group B Streptococcal LEEP derived Proteins in Protein vaccination experiments.****Expression of proteins**

20

Prioritised genes ie, those selected on the basis of predicted expression features as deduced from sequence characteristics (as described in Figure 1), were cloned and expressed as recombinant proteins using the pET system (Novagen, Inc., Madison, WI) utilising *Escherichia coli* as a host. Target genes were cloned into the pET28b(+) plasmid expression vector. The pET28b(+) vector is designed for high level expression and purification of target proteins. This vector carries a T7 promoter for transcription of a target gene, followed by an N-terminal His•Tag<sup>®</sup>/thrombin/T7•Tag<sup>®</sup> configuration, a multi-cloning site containing unique restriction enzyme sites for cloning purposes, and an optional C-terminal His•Tag sequence. The vector also carries a kanamycin resistance gene for selection purposes and for maintaining target gene expression (pET System Manual, 8<sup>th</sup> edition, Novagen).

30

### Preparation of protein vaccines

Oligonucleotide primers were designed for each individual target gene derived using the LEEP system unless stated otherwise. Each gene was examined thoroughly. Where possible primers were designed so that they would target that part of the gene predicted to encode only the mature portion of the protein (APPENDIX II). It is hoped that expressing those corresponding to the predicted mature protein only, might facilitate its correct folding when finally expressed *in vitro*. Oligonucleotide primers were designed so that sequences, encoding the putative N-terminal signal peptide of the target protein, would not be included in the final amplification product to be cloned pET28b(+). The signal peptide directs the polypeptide precursor to the cell membrane via the protein export pathway where it is normally cleaved off by signal peptidase I (or signal peptidase II if a lipoprotein). Hence the signal peptide would not be expected to form any part of the mature target protein, whether it be displayed on the bacterium's surface or secreted. For this purpose, classical signal peptides and their cleavage sites were predicted using the DNA Strider™ Program (CEA, France) and the SignalP V1.1 program, which predicts the presence and location of signal peptide cleavage sites in amino acid sequences from different organisms (Nielsen *et al.*, *Protein Engineering* 10: 1-6 (1997)). Where a N-terminal leader peptide sequence was not obvious, primers were designed to include the whole of the gene sequence for cloning and expression.

All oligonucleotide primers were designed to incorporate appropriate restriction enzyme sites to facilitate cloning into the pcDNA3.1 MCS region (APPENDIX II). Forward primers included an *Nco* I (5'-ccatgg-3') or *Nhe* I (5'-gctagc-3') restriction enzyme site and an 'ATG' start codon in-frame with the target gene open reading frame (orf). All reverse primers included a *Not* I restriction enzyme site 5' - gcggccgc-3' and were designed so that the target gene could be expressed in frame with the C-terminal His•Tag (i.e. the stop codon of the target gene was not

included). Using the *Nco* I and *Not* I, allowed the removal of the N-terminal His•Tag<sup>®</sup>, thrombin and T7•Tag<sup>®</sup> DNA sequences. At the same time target genes were cloned immediately downstream of a highly efficient ribosome binding site (from the phage T7 major capsid protein), to facilitate high level  
5 expression/translation of the target gene by T7 RNA polymerase, and subsequent purification by means of the C-terminal His•Tag. All target gene-specific forward and reverse primers were designed with compatible melting temperatures to facilitate their amplification.

All gene targets were amplified by PCR from *S. agalactiae* genomic DNA template  
10 using Vent DNA polymerase (NEB) using conditions recommended by the manufacturer. A typical amplification reaction involved an initial denaturation step at 95°C for 2 minutes followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at the appropriate melting temperature for 30 seconds, and extension at 72°C for 1 minute (1 minute per kilobase of DNA being amplified). This was  
15 followed by a final extension period at 72°C for 10 minutes. All PCR amplified products were extracted once with phenol:chloroform (2:1:1) and once with chloroform (1:1) and ethanol precipitated. Specific DNA fragments were isolated from agarose gels using the QIAquick Gel Extraction Kit (Qiagen). Purified target gene DNA amplicons were then digested *Nco* I (or *Nhe* I) and *Not* I restriction  
20 enzymes, and cloned into *Nco* I and *Not* I digested pET28b(+) plasmid vector using *E. coli* DH5α or *E. coli* BL21 (DE3) as a host. Successful cloning and maintenance of genes was confirmed by restriction mapping.

#### **Determination of target protein expression and solubility**

25 Glycerol stocks of *E. coli* BL21 DE3 pET28b(+) strains expressing recombinant proteins were used to inoculate 10 ml Luria broth containing Kanamycin (30 µg/ml ) which were grown overnight at 37°C with vigorous shaking (300 rpm).

A 20-40 ml Luria broth containing Kanamycin (30  $\mu$ g/ml) was inoculated with 1:100 dilution of the overnight culture from step 1 and grown at 37°C with vigorous shaking (300 rpm). When the culture reached an OD<sub>600</sub> of between 0.6 and 1.0, IPTG was added to a final concentration of 1mM. Typically cultures were induced  
5 for 3 hours. Cells were then harvested by centrifugation at 7000 g for 10 min. The cell pellet was then resuspended in 1/10 volume of lysis buffer (50mM NaH<sub>2</sub>PO<sub>4</sub>, pH.8.0; 300mM NaCl; 10mM imidazole; 10% glycerol). Lysozyme was then added to a final concentration of 1mg/ml, and the suspension was incubated on ice for 30 min. The suspension was then sonicated on ice (six 10-sec bursts at 200-300 W with  
10 a 10-sec cooling period. The lysate was then centrifuged at 10,000g for 20 min. The supernatant (containing soluble protein) was transferred to a sterile 2 ml eppendorf. The pellet was resuspended in 2 ml of solubilisation buffer (8 M Urea; 50mM NaH<sub>2</sub>PO<sub>4</sub>, pH.8.0; 300mM NaCl; 10% glycerol). This suspension contained the insoluble protein fraction. Aliquots from both the soluble and insoluble fractions  
15 were transferred to new eppendorfs. The protein samples were denatured by adding an equal volume of 2x SDS-PAGE buffer and heating at 95°C for 5 min. Denatured extract samples were then analysed by SDS-PAGE to determine target gene expression and solubility.

**Large scale expression of recombinant target proteins**

5 Glycerol stocks of *E. coli* BL21 DE3 pet28b(+) strains expressing recombinant proteins were used to inoculate 10 ml Luria broth containing Kanamycin ( 30  $\mu$ g/ml ) which were grown overnight at 37°C with vigorous shaking (300 rpm). 5 ml of an overnight culture of a recombinant strain was used to inoculate a 250 ml Luria broth containing kanamycin (30  $\mu$ g/ml) which was grown at 37°C with vigorous shaking (300 rpm). When the culture reached an OD<sub>600</sub> of between 0.6 and 1.0, IPTG was  
10 added to a final concentration of 1mM. Typically, cultures were induced for 3 hours. Cultures were then centrifuged to a pellet and stored frozen at -20°C.

**Purification of target antigens.**

15 Ni-NTA agarose (Qiagen LTD, West Sussex, UK; Cat. No. 30210) was used to purify the His-Tagged recombinant proteins. The 6xHis affinity tag which was expressed in frame with the target proteins in pET28b(+), facilitates binding to Ni-NTA. Ni-NTA offers high binding capacity (with minimal non-specific binding) and can bind 5-10 mg of 6xHis-tagged protein per ml of resin. The 6xHis-tag is poorly  
20 immunogenic, and at pH 8.0, the tag is small, uncharged and therefore does not generally interfere with the structure and function of the protein (The QIAexpressionist, Qiagen Handbook, March 1999).

NOTE: All the proteins (LEEP-derived, unless stated otherwise) described here were  
25 purified under denaturing conditions except ID-65. ID-65 was prepared and purified under native conditions.

**Purification under native conditions**

30 The frozen pellet was allowed to thaw on ice for 15 minutes and then resuspended in 10 ml of lysis buffer (50mM NaH<sub>2</sub>PO<sub>4</sub>, pH.8.0; 300mM NaCl;10mM imidazole;

10% glycerol). Lysozyme was then added to a final concentration of 1mg/ml, and the suspension was incubated on ice for 30 min. The suspension was then sonicated on ice (six 10-sec bursts at 200-300 W with a 10-sec cooling period). Dnase I (5  $\mu$ g/ml) was then added to the lysate, which was then incubated on ice for 10-15 min.

5 The lysate was then centrifuged at 10,000 rpm for 20 min at 4°C to pellet cell debris. The clear lysate supernatant was then loaded into a polypropylene column (Qiagen; Cat. No. 34964), bottom cap attached. 1.5 ml of 50% Ni-NTA was then added, the column sealed and the suspension was allowed to mix gently using a rotating wheel for 1-2 hours at 4°C. The column containing the lysate/Ni-NTA mix was then

10 placed upright using a retort stand, and the Ni-NTA was allowed to settle. The bottom cap was removed and the lysate was allowed to flow through. The column was then washed with three to six 4 ml volumes of wash buffer (50mM NaH<sub>2</sub>PO<sub>4</sub>, pH.8.0; 300mM NaCl; 20mM imidazole; 10% glycerol). The protein was then eluted in 0.5 ml aliquots of elution buffer (50mM NaH<sub>2</sub>PO<sub>4</sub>, pH.8.0; 300mM

15 NaCl; 500mM imidazole; 10% glycerol). Eluate fractions were then analysed by SDS-PAGE and those containing the protein were pooled and dialysed against a PBS (pH 7.0)-glycerol (10%) solution.

#### **Purification and refolding under denaturing conditions**

20 The frozen pellet was allowed to thaw on ice for 15 minutes and then resuspended in 10 ml of buffer containing 8 M Urea, 300 mM NaCl, 10% glycerol, 0.1 M NaH<sub>2</sub>PO<sub>4</sub>, pH.8.0, and 10 mM imidazole. The cells were then lysed by gentle vortexing for 1 hour at room temperature. The lysate was then centrifuged at

25 10,000g for 20 minutes to pellet cellular debris. The clear lysate supernatant was then loaded into a polypropylene column (Qiagen; Cat. No. 34964), bottom cap attached. 1.5 ml of 50% Ni-NTA slurry was then added, the column sealed and the suspension was allowed to mix gently using a rotating wheel for 1-2 hours at room

- temperature. The column containing the lysate/Ni-NTA mix was then placed upright using a retort stand, and the Ni-NTA was allowed to settle. The bottom cap was removed and the lysate was allowed to flow through. The column was then washed with 4-8 ml of buffer containing 8 M Urea, 300 mM NaCl, 10% glycerol, 0.1 M NaH<sub>2</sub>PO<sub>4</sub>, pH 8.0, and 10 mM imidazole. The resin was then washed with a gradient of 6 to 0 M in a buffer containing 0.1 M NaH<sub>2</sub>PO<sub>4</sub>, pH.8.0, 300 mM NaCl and 10% glycerol to facilitate the slow removal of urea and gradual refolding of target protein. The resin was then washed with a buffer containing 0.1 M NaH<sub>2</sub>PO<sub>4</sub>, pH 7.0, 500 mM NaCl and 10% glycerol. The recombinant protein was then eluted in 0.5 ml aliquots with 500 mM Imidazole in 0.1 mM NaH<sub>2</sub>PO<sub>4</sub>, pH 7.0, 500 mM NaCl and 10% glycerol. The fractions were analysed on SDS-PAGE and those containing the protein were pooled and dialysed against a PBS (pH 7.0)-glycerol (10%) solution.
- All purified proteins were analysed by SDS-PAGE, as shown in Figures 5, 6 and 7, prior to their use as antigens in immunisation and vaccination experiments.

### Protein Vaccinations

- Vaccines were composed of the target protein in phosphate buffered saline/10% glycerol and mixed with aluminium hydroxide (alum) (Imject<sup>®</sup> Alum, Pierce, Rockford, Ill.). Each dose (unless otherwise stated) of vaccine contained 25 µg of purified protein in 50 µl of PBS/10% glycerol, mixed with 50 µl of alum. Groups of 6-8 CBA/ca mice (Harlan, UK) were immunised subcutaneously with the vaccines and again 4 weeks later. A control group received 100 µl dose of PBS/10% glycerol with alum. All vaccinated groups consisted of 6 mice. Mice were challenged at 7 weeks (unless otherwise stated). Mice were injected intraperitoneally (i.p.) with between 2.5-5 X 10<sup>6</sup> bacteria diluted in 0.5 ml Todd-Hewitt broth. Deaths were recorded daily for 7 days. The challenged mice were observed daily for signs of illness. Typical symptoms in an appropriate order included piloerection, an

increasingly hunched posture, discharge from eyes, increased lethargy and reluctance to move which was often the result of apparent paralysis in the lower body/hind leg region. The latter symptoms usually coincided with the development of a moribund state at which stage the mice were culled to prevent further suffering. These mice were deemed to be very close to death, and the time of culling was used to determine a survival time for statistical analysis. Where mice were found dead, a survival time was calculated by averaging the time when a particular mouse was last observed alive and the time when found dead, in order to determine a more accurate time of death.

#### **Analysis of antibody responses**

Mice (6 per group) were immunised with two doses of vaccine with a four week interval. Mice were tail bled at 3 weeks and 6 weeks post primary vaccination to obtain sera. Total Immunoglobulin G (IgG) titres to the vaccine protein component in sera were determined by enzyme-linked immunosorbent assay (ELISA), using the original purified protein as the coating antigen.

#### **Standard ELISA protocol**

##### **Solutions**

##### **Carbonate/bicarbonate buffer, pH 9.8**

0.80g  $\text{Na}_2\text{CO}_3$

1.46g  $\text{NaHCO}_3$

pH to 9.6 using HCl

Add distilled water ( $\text{dH}_2\text{O}$ ) to a final volume of 500ml.

#### **n-NITROPHENYL PHOSPHATE SUBSTRATE**

**Diethanolamine Buffer, pH 9.8**

48.5 ml diethanolamine

pH to 9.8 using 1M HCl

Add dH<sub>2</sub>O to a final volume of 500ml

5

NOTE: ELISAs were optimised for each protein submitted for immunisation.

**PROTOCOL**

- 10 1. ELISA plates (Greiner labortechnik 96 well plates: Cat. No. 655061) with an appropriate concentration of recombinant protein diluted in carbonate/bicarbonate buffer (50  $\mu$ l/well). Cover plates with plastic or foil and leave overnight at 4°C.
2. Quickly wash plates twice in a tub/container containing PBS/0.05% Tween-20 and then pat dry.
- 15 3. Block plates with 3% BSA in PBS/Tween (100 $\mu$ l /well) for 1 hour at room temperature.
4. Wash the plates 3 times PBS/Tween as before and pat dry as before.
5. Apply (primary antibody) protein-specific antiserum (50 $\mu$ l/well) diluted from 1/50 in a doubling dilution series in PBS/Tween and incubate at room temperature for 90 minutes.
- 20 6. Wash plates as before (3 times quickly), followed up by 2 X 3 minute soaks (in PBS/Tween)
7. Apply diluted secondary antibody alkaline phosphatase conjugate. For anti-mouse Total IgG alkaline phosphatase conjugate (Goat Anti-Mouse IgG-AP, Southern Biotechnology Associates, Birmingham, AL. Cat. No. 1030-04) dilute 1/3000 in
- 25 PBS/Tween and apply 50  $\mu$ l per well and incubate at room temperature for 90 minutes.
8. Wash plates as in step 6.

9. Apply substrate. Dissolve one 5mg tablet of nitrophenyl phosphate (Sigma:kept in freezer) in 5ml of diethanolamine buffer. Apply 100  $\mu$ l per well. Cover with foil (a light-sensitive reaction) and leave at room temperature for 30 minutes. Read Optical densities (OD) at a wavelength of 405nm.
- 5 10. Plot curves of OD Vs dilution (log scale). Calculate end-point titres as the dilution giving the same OD as the mean of the OD obtained from the wells containing the 1/50 dilution of pre-immune serum.

10

15

**ELISA Plate format**

|         |               |           |           |           |           |            |            |            |                 |                 |                 |
|---------|---------------|-----------|-----------|-----------|-----------|------------|------------|------------|-----------------|-----------------|-----------------|
| 2°      | 1/50          | 1/10<br>0 | 1/20<br>0 | 1/40<br>0 | 1/80<br>0 | 1/160<br>0 | 1/32<br>00 | 1/640<br>0 | 1/<br>1280<br>0 | 1<br>/256<br>00 | 1/<br>5120<br>0 |
| 1°      | Dupl<br>icate |           |           |           |           |            |            |            |                 |                 |                 |
| Pr<br>e |               |           |           |           |           |            |            |            |                 |                 |                 |
| Pr<br>e |               |           |           |           |           |            |            |            |                 |                 |                 |
| Pr<br>e |               |           |           |           |           |            |            |            |                 |                 |                 |
|         |               |           |           |           |           |            |            |            |                 |                 |                 |
|         |               |           |           |           |           |            |            |            |                 |                 |                 |
|         |               |           |           |           |           |            |            |            |                 |                 |                 |

**Table Summary**

5     **Pre**     Replicate wells of pooled pre-inoculation serum (50µl per well) diluted to 1/50 are included on every plate in order for end point titres to be calculated.

2°     Is a blank control well to which no secondary antibody conjugate is applied. PBS/Tween by itself is applied instead

10    1°     Is a blank control well to which no primary antibody is applied. PBS/Tween by itself is applied instead

**Duplicate**     Each serum is analysed in duplicate

The dilution series used is indicated (see first row of table). Beginning with a 1/50 dilution, sera are diluted two-fold in PBS/Tween in doubling dilution series as indicated.

15

**Protein Immunisation data****ID-65 and ID-83**

The ID-65 and ID-83 vaccines were composed of the target proteins in phosphate buffered saline/10% glycerol mixed with aluminium hydroxide (alum)

(Imject<sup>®</sup> Alum, Pierce, Rockford, Ill.). Each dose of vaccine contained 20  $\mu$ g of purified protein in 100  $\mu$ l of PBS/10% glycerol, mixed with 50  $\mu$ l of alum. A group of 6-8 week old CBA/ca mice (Harlan, UK) were immunised subcutaneously with the ID-65 and ID-83 vaccine and again 4 weeks later. A control group received a  
5 150  $\mu$ l dose of PBS/10% glycerol (2:1) with alum. All groups consisted of 6 mice. Mice were tail bled at 5 weeks post primary vaccination to obtain sera. The presence of total Immunoglobulin G (IgG) antibodies to the ID-65 and ID-83 protein in sera was determined by enzyme-linked immunosorbent assay (ELISA), using the purified protein as the coating antigen. ELISA was also performed using sera obtained at 6  
10 weeks post-primary vaccination from the PBS/10% glycerol immunised control group.

NOTE: ELISA plates were coated with the ID-65 or ID-83 proteins at a concentration of 1  $\mu$ g/ml.

15

#### **Protein Vaccination -ELISA results for ID-65 and ID-83**

Mice (6 per group) were immunised with two doses of the ID-65 and ID-83 vaccines with a four week interval. Mice were tail bled at 5 weeks post primary  
20 vaccination to obtain sera. The Immunoglobulin G (IgG) titres to the vaccine protein component in sera were determined by enzyme-linked immunosorbent assay (ELISA), using the purified ID-65 and ID-83 proteins as the coating antigen. Subsequent to optimisation, ELISA plates were coated at a concentration 1 $\mu$ g/ml for both the purified ID-65 and ID-93 proteins. Total IgG titres were measured against  
25 pre-immune serum (1/50 dilution). The results are shown in Table 2 and graphically in Figure 8.

30

Table 2

| Serum (Group)                                    | <u>ID-65 + Alum</u><br>(n=6) | <u>PBS + Alum</u><br>(n=6) | <u>ID-83 + Alum</u><br>(n=6) | <u>PBS + Alum</u><br>(n=6) |
|--|------------------------------|----------------------------|------------------------------|----------------------------|
| Coating antigen                                  | <u>ID-65</u>                 |                            | <u>ID-83</u>                 |                            |
| Bleed  | <u>5 weeks</u>               | <u>5 weeks</u>             | <u>5 weeks</u>               | <u>5 weeks</u>             |
| <u>Total IgG</u><br><u>Titres</u><br>(mouse 1-6) | 7535763                      | 965                        | 82081                        | 61                         |
|  | 1557649                      | 90                         | 50027                        | 50                         |
|  | 3319737                      | 108                        | 154670                       | 80                         |
|  | 1832259                      | 176                        | 57901                        | 96                         |
|  | 8794360                      | 371                        | 66497                        | 125                        |
|  | 1445728                      | 0                          | 49928                        | 0                          |
| <u>Average</u>                                   | 4080916                      | 285                        | 76851                        | 69                         |
| <u>Standard Deviation</u>                        | 3258818                      | 355                        | 39985                        | 43                         |

5

**Protein Immunisation and Challenge data (ID-93)****ID-93**

10 The ID-93 vaccine was composed of the target ID-93 protein in phosphate buffered saline/10% glycerol mixed with aluminium hydroxide (alum) (Imject® Alum, Pierce, Rockford, Ill.). Each dose of vaccine contained 25 µg of purified protein in 100 µl of PBS/10% glycerol, mixed with 100 µl of alum. A group of 6-8 week old CBA/ca mice (Harlan, UK) were immunised subcutaneously with the ID-93 vaccine and again 4 weeks later. A control group received PBS/10% glycerol with alum. Both

15 groups consisted of 6 mice. Mice were challenged at 7 weeks (unless otherwise stated). Mice were injected intraperitoneally (i.p.) with  $5 \times 10^6$  bacteria diluted in

0.5 ml Todd-Hewitt broth. The challenged mice were observed daily for signs of illness. Deaths were recorded daily for 7 days. Survival data are shown in Table 3 and graphically in Figure 9.

- 5 Mice were tail bled at 3 weeks and 6 weeks post primary vaccination to obtain sera. The presence of total Immunoglobulin G (IgG) antibodies to the ID-93 protein in sera was determined by enzyme-linked immunosorbent assay (ELISA), using the pure ID-93 protein as the coating antigen. ELISA was also performed using sera obtained at 6 weeks post-primary vaccination from the PBS/10% glycerol immunised control group.
- 10

Note: ELISA plates were coated with the ID-93 protein at a concentration of 1  $\mu\text{g/ml}$ .

15 Table 3  
ID-93 protein immunisation and GBS challenge experiment

**Statistical analysis of Survival Times**

| Group                         | <u>PBS + Alum</u> | <u>ID-93 + Alum</u> |
|-------------------------------|-------------------|---------------------|
| <u>Survival Times (hours)</u> | 22.37             | 29.37               |
|                               | 22.37             | 35.12               |
|                               | 15.37             | 32.62               |
|                               | 28.03             | 32.62               |
|                               | 29.53             | 37.12               |
|                               | 26.53             | 27.87               |
| <b>Mean</b>                   | 24.03             | 32.45               |
| <b>sd</b>                     | 5.16              | 3.45                |
| <b>p value</b>                |                   | 0.01                |

20

**p value** refers to statistical significance when compared to unvaccinated controls.

### Comment

#### 5 ID-93 (RS-70)

Mice immunised with the ID-93-Alum vaccine exhibited significantly longer survival times when compared with the PBS-Alum control group.

10 (Statistical Significance was determined by the Mann-Whitney U test using a 95 % confidence level ( $p > 0.05$ ).

#### Protein Vaccination -ELISA results for ID-93

15 Mice (6 per group) were immunised with two doses of the ID-93 vaccine with a four week interval. Mice were tail bled at 3 weeks and 6 weeks post primary vaccination to obtain sera. The Immunoglobulin G (IgG) titres to the vaccine protein component in sera were determined by enzyme-linked immunosorbent assay (ELISA), using the purified ID-93 protein as the coating antigen. Subsequent to optimisation, ELISA plates were coated with the purified ID-93 protein at a concentration of 1  $\mu\text{g/ml}$ .  
20 Total IgG titres were measured against pre-immune serum (1/50 dilution). The results are shown in Table 4 and graphically in Figure 10.

Table 4

| Serum Group  | <u>ID-93 + Alum(n=6)</u> |                | <u>PBS/10%glycerol (n=6)</u><br><u>(control)</u> |                |
|--|--------------------------|----------------|--|----------------|
| Coating antigen  | <u>ID-93</u>             | <u>ID-93</u>   | <u>ID-93</u>                                     | <u>ID-93</u>   |
| Bleed  | <u>3 weeks</u>           | <u>6 weeks</u> | <u>3 weeks</u>                                   | <u>6 weeks</u> |
| <u>Total IgG</u><br><u>Titres</u><br><u>(mouse 1-</u><br><u>6)</u> | 87196                    | 3000000        | 39   | 100            |
|  | 99544                    | 8000000        | 31   | 16             |
|  | 19620                    | 2000000        | 31   | 79             |
|  | 34724                    | 10000000       | 59   | 48             |
|  | 59990                    | 10000000       | 24   | 328            |
|  | 30041                    | 4000000        | 13   | 40             |
| <u>Average</u>   | 55186                    | 6166667        | 33   | 102            |
| <u>Standard error</u>  | 32654                    | 3600926        | 15   | 115            |

5

#### **Protein Immunisation data** **ID-89 and ID-96**

10 The ID-89 and ID-96 vaccines were composed of the target proteins in phosphate buffered saline/10% glycerol mixed with TitreMax Gold adjuvant (Sigma, Missouri, USA) according to the manufacturers instructions. The ID-89 vaccine contained 25  $\mu$ g of purified protein 50  $\mu$ l of PBS/10% glycerol, mixed with 50  $\mu$ l of TitreMax Gold. The ID-96 vaccine contained 12.5  $\mu$ g of purified protein 50  $\mu$ l of PBS/10% glycerol, mixed with 50  $\mu$ l of TitreMax Gold. Groups of 6-8 week old CBA/ca mice

15 (Harlan, UK) were immunised subcutaneously with the ID-89 and ID-96 vaccines and again 4 weeks later. A control group received a 100  $\mu$ l dose PBS/10% glycerol with TitreMax Gold (1:1). Both groups consisted of 6 mice. Mice were tail bled at 3

weeks and 6 weeks post primary vaccination to obtain sera. The presence of total Immunoglobulin G (IgG) antibodies to the ID-65 and ID-83 protein in sera was determined by enzyme-linked immunosorbent assay (ELISA), using the purified protein as the coating antigen. ELISA was also performed using sera obtained at 3 weeks and 6 weeks post-primary vaccination from the PBS/10% glycerol immunised control group.

Note: ELISA plates were coated with the ID-89 or ID-96 proteins at a concentration of 1  $\mu$ g/ml and 3  $\mu$ g/ml respectively.

#### **Protein Vaccination -ELISA results for ID-89 and ID-96**

Mice (6 per group) were immunised with two doses of the ID-89 and ID-96 vaccines with a four week interval. Mice were tail bled at 3 weeks and 6 weeks post primary vaccination to obtain sera. The Immunoglobulin G (IgG) titres to the vaccine protein component in sera were determined by enzyme-linked immunosorbent assay (ELISA), using the purified ID-65 and ID-83 proteins as the coating antigen. Subsequent to optimisation, ELISA plates were coated with purified ID-89 and ID-96 protein at a concentration 1 $\mu$ g/ml and 3  $\mu$ g/ml respectively. Total IgG titres were measured against pre-immune serum (1/50 dilution). ELISA was also performed on both proteins using sera obtained at 3 weeks and 6 weeks post-primary vaccination from the PBS/10% glycerol immunised control group. Results are shown in tables 5a and 5b and graphically in Figure 11.

Table 5a

| Serum  | ID-89 + TitreMax Gold (n=6) |                | ID-96 + TitreMax Gold(n=6) |                |
|--|-----------------------------|----------------|----------------------------|----------------|
| Coating antigen  | ID-89                       |                | ID-96                      |                |
| Bleed  | <u>3 weeks</u>              | <u>6 weeks</u> | <u>3 weeks</u>             | <u>6 weeks</u> |
| <u>Total IgG</u><br><u>Titres</u><br><u>(mouse 1-</u><br><u>6)</u> | 146940                      | 1000000        | 190371                     | 10000000       |
|  | 89672                       | 1000000        | 212505                     | 10000000       |
|  | 173532                      | 2000000        | 167613                     | 5000000        |
|  | 85161                       | 751210         | 110378                     | 5000000        |
|  | 88956                       | 551281         | 142614                     | 1000000        |
|  | 27880                       | 2000000        | 191085                     | 1000000        |
| Average  | 102024                      | 1217082        | 169094                     | 5333333        |
| Standard Deviation   | 51451                       | 629364         | 37341                      | 4033196        |

Table 5b

| Serum                               | <u>PBS/10% glycerol (n=6)</u> |                | <u>PBS/10% glycerol (n=6)</u> |                |
|-------------------------------------|-------------------------------|----------------|-------------------------------|----------------|
| Coating protein                     | <u>ID-89</u>                  |                | <u>ID-96</u>                  |                |
| Bleed                               | <u>3 weeks</u>                | <u>6 weeks</u> | <u>3 weeks</u>                | <u>6 weeks</u> |
| <u>Total IgG Titres (mouse 1-6)</u> | 3                             | 7              | 33                            | 31             |
|                                     | 8                             | 18             | 77                            | 62             |
|                                     | 29                            | 31             | 77                            | 1              |
|                                     | 34                            | 4              | 52                            | 29             |
|                                     | 0                             | 2              | 125                           | 31             |
|                                     | 5                             | 1              | 113                           | 0              |
| Average                             | 13                            | 11             | 80                            | 26             |
| Standard deviation                  | 15                            | 12             | 35                            | 23             |

**Example 4****5 Conservation and variability of candidate vaccine antigen genes among different isolates of Group B Streptococci**

An initial Southern blot analysis was carried out to determine cross-serotype conservation of novel Group B Streptococcal genes isolated using the LEEP system unless stated otherwise. Analysing the serotype distribution of a target gene will also  
10 determine their potential use as antigen components in a GBS vaccine. The Group B Streptococcal strains whose DNA was analysed as part of this study are listed in APPENDIX III

**15 Amplification and labelling of specific target genes as DNA probes for Southern blot analysis.**

Oligonucleotide primers were designed for each individual gene of interest derived using the LEEP system unless stated otherwise. The same primers already described in APPENDIX II were used to amplify corresponding gene-specific DNA probes. Specific gene targets were amplified by PCR using Vent DNA polymerase (NEB)  
20 according to the manufacturers instructions. Typical reactions were carried out in a 100  $\mu$ l volume containing 50 ng of GBS template DNA, a one tenth volume of enzyme reaction buffer, 1  $\mu$ M of each primer, 250  $\mu$ M of each dNTP and 2 units of Vent DNA polymerase. A typical reaction contained an initial 2 minute denaturation at 95°C, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at  
25 the appropriate melting temperature for 30 seconds, and extension at 72°C for 1 minute (1 minute per kilobase of DNA being amplified). The annealing temperature was determined by the lower melting temperature of the two oligonucleotide primers. The reaction was concluded with a final extension period of 10 minutes at 72°C.

All PCR amplified products were extracted once with phenol chloroform (2:1:1) and once with chloroform (1:1) and ethanol precipitated. Specific DNA fragments were isolated from agarose gels using the QIAquick Gel Extraction Kit (Qiagen). For use as DNA probes, purified amplified gene DNA fragments were labelled with digoxigenin using the DIG Nucleic Acid Labelling Kit (Boehringer Mannheim) according to the manufacturer's instructions.

#### **Southern blot hybridisation analysis of Group B Streptococcal genomic DNA**

Genomic DNA had previously been isolated from all strains of Group B Streptococci which were investigated for conservation of LEEP-derived (unless stated otherwise) gene targets. Appropriate DNA concentrations were digested using either *Hin* DIII or *Eco* RI restriction enzymes (NEB) according to manufacturer instructions and analysed by agarose gel electrophoresis. Following agarose gel electrophoresis of DNA samples, the gel was denatured in 0.25M HCl for 20 minutes and DNA was transferred onto Hybond<sup>TM</sup> N<sup>+</sup> membrane (Amersham) by overnight capillary blotting. The method is essentially as described in Sambrook *et al.* (1989) using Whatman 3MM wicks on a platform over a reservoir of 0.4M NaOH. After transfer, the filter was washed briefly in 2x SSC and stored at 4°C in Saran wrap (Dow chemical company).

Filters were prehybridised, hybridised with the digoxigenin labelled DNA probes and washed using conditions recommended by Boehringer Mannheim when using their DIG Nucleic Acid Detection Kit. Filters were prehybridised at 68°C for one hour in hybridisation buffer (1% w/v supplied blocking reagent, 5x SSC, 0.1% v/v N-lauryl sarcosine, 0.02% v/v sodium dodecyl sulphate[SDS]). The digoxigenin labelled DNA probe was denatured at 99.9°C for 10 minutes before being added to the hybridisation buffer. Hybridisation was allowed to proceed overnight in a rotating Hybaid tube in a Hybaid Mini-hybridisation oven. Unbound probe was removed by washing the filter twice with 2x SSC- 0.1% SDS for 5 minutes at room

temperature. For increased stringency filters were then washed twice with 0.1x SSC-0.1% SDS for 15 minutes at 68°C. The DIG Nucleic Acid Detection Kit (Boehringer Mannheim) was used to immunologically detect specifically bound digoxigenin labelled DNA probes.

5

### Results of Southern blot analysis

Unless otherwise stated, all genomic digests and their corresponding Southern blots followed an identical lane order as described in Table 6 below.

10

**Table 6**

| Lane     | 1              | 2   | 3    | 4    | 5    | 6      | 7       |
|----------|----------------|-----|------|------|------|--------|---------|
| Strain   | 1 kb molecular | 515 | A909 | SB35 | H36B | 18RS21 | 1954/92 |
| Serotype | Weight Marker  | Ia  | Ia   | Ib   | Ib   | II     | II      |

| Lane     | 8       | 9       | 10   | 11   | 12      | 13   | 14      |
|----------|---------|---------|------|------|---------|------|---------|
| Strain   | 118/158 | 97/0057 | BS30 | M781 | 97/0099 | 3139 | 1169-NT |
| Serotype | II      | II      | III  | III  | III     | IV   | V       |

| Lane     | 15    | 16   | 17   | 18                    | 19                              | 20             |
|----------|-------|------|------|-----------------------|---------------------------------|----------------|
| Strain   | GBS 6 | 7271 | JM9  | Group A Streptococcus | <i>Streptococcus pneumoniae</i> | 1 kb molecular |
| Serotype | VI    | VII  | VIII | —                     | 14                              | Weight Marker  |

15

For comparative purposes, it was decided to analyse the serotype distribution of the GBS *rib* gene, which encodes the known protective immunogen Rib. Rib has previously been shown to be present in serotype III and some strains of serotype II but not in serotypes Ia or Ib (Stalhammar-Carlemalm *et al.*, *J. Exp. Med.* 177: 1593-1603 (1993)).

Confirmation of this pattern would not only give increased confidence in interpreting subsequent results, it would also determine if a *rib* gene homologue was present in the remaining GBS serotypes being investigated here. Primers designed for the amplification of *rib* for use as a gene probe in Southern blot analysis are described in APPENDIX II.

Table 7 – Lane order for Figure 12 (*rib* gene Southern blot analysis)

| Lane     | 1              | 2   | 3    | 4    | 5    | 6      | 7       |
|----------|----------------|-----|------|------|------|--------|---------|
| strain   | 1 kb molecular | 515 | A909 | SB35 | H36B | 18RS21 | 1954/92 |
| serotype | Weight Marker  | Ia  | Ia   | Ib   | Ib   | II     | II      |

| Lane     | 8       | 9       | 10    | 11   | 12   | 13      | 14   |
|----------|---------|---------|-------|------|------|---------|------|
| strain   | 118/158 | 97/0057 | BM110 | BS30 | M781 | 97/0099 | 3139 |
| serotype | II      | II      | III   | III  | III  | III     | IV   |

| Lane     | 15      | 16    | 17   | 18   | 19                       | 20                              |
|----------|---------|-------|------|------|--------------------------|---------------------------------|
| strain   | 1169-NT | GBS 6 | 7271 | JM9  | Group A<br>Streptococcus | <i>Streptococcus pneumoniae</i> |
| serotype | V       | VI    | VII  | VIII | —                        | 14                              |

### **Rib (Figure 12) Comment**

5 The Southern blot analysis shown in Figure 12 indicates that the *rib* gene is not conserved across all GBS serotypes. *rib* appears to be absent from all serotype Ia and Ib strains (lanes 2 to 5) and from strains 118/158 and 97/0057 of serotype II (lanes 8 and 9). However, *rib* would appear to present in strains 18RS21 and 1954/92 of serotype II (lanes 6 and 7) and in all strains of serotype III (lanes 10 to 13). This is in agreement with previously published data (Stalhammar-Carlemalm *et al.*, 1993 [*supra*]). *rib* would also appear to be present in strains representing serotypes VII and VII (lanes 17 and 18) but was absent from strains representing serotypes IV, V and V (lanes 14 to 16) as well as the control strains (lanes 19 and 20). The *rib* gene probe did hybridise with lower intensity to genomic DNA fragments from strains representing serotypes Ia, Ib, IV, VI, VII and serotype II strains 118/158 and 97/0057. This may indicate the presence of a gene in these strains with a lower level of homology to *rib*. These hybridising DNA fragments may contain a homologue of the GBS *bca* gene encoding the Ca protein antigen which has been shown to be closely homologous to the Rib protein (Wastfelt *et al.*, *J. Biol. Chem.* 271:18892-18897 (1996)). If this is the case, it would be in agreement with previous work which showed all strains of serotypes Ia, Ib, II and III to be positive for one the two proteins (Stalhammar-Carlemalm *et al.*, 1993 [*supra*]). However, the apparent variable distribution of the *rib* gene amongst different GBS

serotypes, makes it a less than ideal candidate for use in a GBS vaccine that is cross-protective against all serotypes.

5

**ID-65 (Figure 13) Comment**

The Southern blot analysis described in Figure 13 indicates that gene ID-65 is conserved across all GBS serotypes. The gene probe hybridised specifically to a *Hin* DIII-digested genomic DNA fragment of approximately 3.0 kb in DNA digests from all GBS representatives, and was absent from both the control strains (lanes 18 and 19). This would suggest that the ID-65 gene is conserved across all GBS serotypes (and strains) at both the gene and locus level. The ID-65 DNA probe also hybridised weakly to the 1.636 bp molecular weight marker (the 1 kb DNA ladder from NEB was used to estimate DNA fragment sizes in all Southern blot analyses).

15

**ID-89 (Figure 14) Comment**

The Southern blot analysis described in Figure 14 indicates that gene ID-89 may not be conserved across all GBS serotypes. A 4.0 kb *Hin*DIII-digested genomic DNA fragment from 12 out of 16 GBS strains hybridised specifically to the ID-89 gene probe. In addition, a 3.25 kb *Hin*DIII-digested genomic DNA fragment from the GBS strain Ib (SB35) [lane 4] also hybridised specifically with the ID-89 gene probe. However, the ID-89 gene probe did not hybridise to digested genomic DNA fragments from strains Ia (515) [lane 2], IV (3139) [lane 13] and V (1169-NT) [lane 14], suggesting that these strains do not possess a ID-89 gene homologue.

25

**ID-93 (Figure 15) Comment**

The Southern blot analysis described in Figure 15 indicates that gene ID-93 is conserved across all GBS serotypes. The gene probe hybridised specifically to a *Hin*

DIII-digested genomic DNA fragment of approximately 3.25 kb in DNA digests from all GBS representatives, and was absent from both the control strains (lanes 18 and 19). This would suggest that the ID-93 gene is conserved across all GBS serotypes (and strains) at both the gene and locus level.

5

**ID-96 (Figure 16) Comment**

The Southern blot analysis described in Figure 16 indicates that gene ID-96 is conserved across all GBS serotypes. The gene probe hybridised specifically to a *Eco* RI-digested genomic DNA fragment of approximately 12.0 kb in DNA digests from all GBS representatives, and was absent from both the control strains (lanes 18 and 19). This would suggest that the ID-96 gene is conserved across all GBS serotypes (and strains) at both the gene and locus level.

10

**APPENDIX I****ID-65**

Forward Primer

5 5' - cggatccgccaccatgGCGGATCAA~~A~~CTACATCGGTTC - 3'

Reverse Primer

5' - ttgcggccgcGTTGGGATAACTAGTCGGTTTAGTCG

Length (including restriction sites) = 1541bp

10 Incorporating 1515bp of gene-specific sequence encoding 505 amino acids of the putative mature protein.

Annealing temperature for PCR amplification = 60°C

Sequence predicted to encode a signal peptide was omitted from amplified product

15 **ID-66**

Forward Primer

5' - cggatccgccaccatgAATCTTTATTTCATAGTACTCCCTTGC - 3'

Reverse Primer

5' - ttgcggccgcAAAATGATCAGTTTGAGGGTAAAAGAG - 3'

20

Length (including restriction sites) = 767bp

Incorporating 747bp of gene-specific sequence encoding 247 amino acids of the putative mature protein.

Annealing temperature for PCR amplification = 60°C

25 Sequence predicted to encode a signal peptide was omitted from amplified product

**APPENDIX II**

ID-65

Forward Primer

5' - catgcatgGCGGATCAAACATCATCGGTTC - 3'

5 Reverse Primer

5' - ttgcggccgcGTTGGGATAACTAGTCGGTTTAGTCG

Length (including restriction sites) = 1534bp

10 Incorporating 1515bp of gene-specific sequence encoding 505 amino acids of the putative mature protein.

Annealing temperature for PCR amplification = 60°C

ID-83

15 Forward Primer

5' - catgcatggcaAAAATAGTAGTACCAGTAATGCCTC - 3'

ReversePrimer

5' - ttgcggccgcCTCTGAAATAGTAATTTGTCCG - 3'

20 Length (including restriction sites) = 626bp

Incorporating 624bp of gene-specific sequence encoding 208 amino acids of the putative mature protein.

Annealing temperature for PCR amplification = 52°C

25

ID-89

Forward Primer

5' - catgcatgggaAAGAAAGCAAATAATGTCAGTCC - 3'

## Reverse Primer

5' – ttgcggccgcATTGGGTGTAAGCATTTTTTC – 3'

Length (including restriction sites) = 990bp

5 Incorporating 969bp of gene-specific sequence encoding 323 amino acids of the putative mature protein.

Annealing temperature for PCR amplification = 54°C

ID-93

## Forward Primer

10 5' – catgccatgggaACTGAGAACTGGTTACATACTAAAG – 3'

## ReversePrimer

5' – ttgcggccgcATTAGCTTTTTCAACAATTTCTC – 3'

Length (including restriction sites) = 759bp

15 Incorporating 744bp of gene-specific sequence encoding 248 amino acids of the putative mature protein.

Annealing temperature for PCR amplification = 51°C

ID-96

20 Forward Primer

5' – ctagctagccgATGTTTGCGTGGGAAAG – 3'

## ReversePrimer

5' – ttgcggccgcATAAGATTTAACAATACCAAGTAATATAGC – 3'

Length (including restriction sites) = 944bp

25 Incorporating 921bp of gene-specific sequence encoding 307 amino acids of the putative mature protein.

Annealing temperature for PCR amplification = 53°C

rib (control)

Forward primer

5' - ggggtaccggccaccATGGCTGAAGTAATTTTCAGGAAGT -3'

5 Reverse primer

5' - cggaattccgTTAATCCTCTTTTTTTCTTAGAAACAGAT

Length (including restriction sites) = 3559bp

10 Incorporating 3531bp of gene-specific sequence encoding 1177 amino acids of the mature protein.

Annealing temperature for PCR amplification = 55°C

**APPENDIX III**

15 Listed below are the details (serotype and strain designation) of Group B Streptococcus strains whose DNA was analysed for gene conservation

|    | SEROTYPE | STRAIN  |
|----|----------|---------|
| 20 | Ia       | 515     |
|    | Ia       | A909    |
|    | Ib       | SB35    |
|    | Ib       | H36B    |
|    | II       | 18RS21  |
| 25 | II       | 1954/92 |
|    | II       | 118/158 |
|    | II       | 97/0057 |
|    | III      | BM110   |
|    | III      | BS30    |
| 30 | III      | M781    |
|    | III      | 97/0099 |
|    | IV       | 3139    |

|      |         |
|------|---------|
| V    | 1169/NT |
| VI   | GBS VI  |
| VII  | 7271    |
| VIII | JM9     |

5

A group A Streptococcal strain (serotype M1, strain NCTC8198) and *Streptococcus pneumoniae* (serotype 14) were also included in the analysis for control purposes.

CLAIMS

1. A Group B Streptococcus polypeptide or protein having a sequence selected  
5 from those described in fig 1, or fragments or derivatives thereof.
2. Derivatives or variants of the proteins, polypeptides, and peptides as claimed  
in claim 1 which show at least 50% identity to those proteins, polypeptides and  
peptides claimed in claim 1.  
10
3. A Group B Streptococcus polypeptide or protein, or derivative or variant  
thereof, as claimed in claim 1 or claim 2 , which is isolated or recombinant.
4. A nucleic molecule comprising or consisting of a sequence which is:  
15
- (i) any of the DNA sequences set out in figure 1 herein or their RNA  
equivalents;
  - (ii) a sequence which is complementary to any of the sequences of (i);
  - (iii) a sequence which codes for the same protein or polypeptide, as those  
20 sequences of (i) or (ii);
  - (iv) a sequence which shows substantial identity with any of those of (i), (ii)  
and (iii); or
  - (v) a sequence which codes for a derivative, or fragment of a nucleic acid  
molecule shown in figure 1.  
25
5. A vector comprising one or nucleic acid molecules as defined in claim 4.

6. A vector as claimed in claim 4 further comprising nucleic acid encoding any one or more of the following: promoters, enhancers, signal sequences, leader sequences, translation start and stop signals, DNA stability controlling regions, or a fusion partner.

5

7. The use of a vector as claimed in claim 5 or claim 6 in the transformation or transfection of a prokaryotic or eukaryotic host.

8. A host cell transformed with a vector as defined in claim 5 or claim 6..

10

9. A process for producing a Group B Streptococcus polypeptide or protein, or derivative or variant thereof, as claimed in claim 1 or claim 2, the process comprising expressing the polypeptide or protein in a host cell as claimed in claim 8.

15

10. An antibody, an affibody, or a derivative thereof which binds to one or more of the proteins, polypeptides, peptides, fragments or derivatives thereof, as defined in any one of claims 1 to 3.

20

11. An immunogenic composition comprising one or more of the proteins, polypeptides, peptides, fragments or derivatives thereof as defined in any one of claims 1 to 3.

25

12. An immunogenic composition as claimed in claim 11 wherein the proteins, polypeptides, peptides, or fragments or derivatives thereof include ID-65 or ID-83, ID-89, ID-93 or ID-96.

13. An immunogenic composition as claimed in claim 11 or claim 12 which is a vaccine.

14. An immunogenic composition comprising one or more of the nucleic acid sequences as defined in claim 4.
- 5 15. An immunogenic composition as claimed in claim 14 wherein the nucleic acid sequences include ID-65 or ID-66.
16. An immunogenic composition as claimed in claim 14 or claim 15 which is a vaccine.
- 10 17. Use of an immunogenic composition as defined in any one of claims 11 to 16 in the preparation of a medicament for the treatment or prophylaxis of Group B Streptococcus infection.
- 15 18. A method of detection of Group B Streptococcus which comprises the step of bringing into contact a sample to be tested with at least one antibody, affibody, or a derivative thereof, as defined in claim 10.
- 20 19. A method of detection of Group B Streptococcus which comprises the step of bringing into contact a sample to be tested with at least one protein, polypeptide, peptide, fragments or derivatives as defined in any one of claims 1 to 3.
- 25 20. A method of detection of Group B Streptococcus which comprises the step of bringing into contact a sample to be tested with at least one nucleic acid molecule as defined in claim 4.
21. A kit for the detection of Group B Streptococcus comprising at least one antibody, affibody, or derivatives thereof as defined in claim 10.

22. A kit for the detection of Group B Streptococcus comprising at least one Group B Streptococcus protein, polypeptide, peptide, fragment or derivative thereof as defined in any one of claims 1 to 3.

5

23. A kit for the detection of Group B Streptococcus comprising at least one nucleic acid molecule as defined in claim 4.

10

24. A method of determining whether a protein, polypeptide, peptide, fragment or derivative thereof as defined in any one of claims 1 to 3 represents a potential anti-microbial target which comprises inactivating said protein and determining whether Group B Streptococcus is still viable.

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## FIG. 1

ID-65

Clone 3-60

GTGTTTATGATGAAAAAAGGACAAGTAAATGATACTAAGCAA  
TCTTACTCTCTACGTAAATATAAATTTGGTTTAGCATCAGTAA  
TTTtagggTCATTcATAATGGTCACAAGTCCTGTTTTGCGGA  
TCAAACtACATCGGTTCAAGTTAATAATCAGACAGGCACTAG  
TGTGGATGCTAATAATCTTCCAATGAGACAAGTGCGTCAAGT  
GTGATTACTTCCAATAATGATAGTGTTCAAGCGTCTGATAAAG  
TTGTAAATAGTCAAAATACGGCAACAAAGGACATTACTACTC  
CTTTAGTAGAGACAAAGCCAATGGTGGAAAAACATTACCTG  
AACAAGGGAATTATGTTTATAGCAAAGAAACCGAGGTGAAAA  
ATACACCTTCAAAATCAGCCCCAGTAGCTTTCTATGCAAAGAA  
AGGTGATAAAGTTTTCTATGACCAAGTATTTAATAAAGATAAT  
GTGAAATGGATTTTCATATAAGTCTTTTGGTGGCGTACGTCGAT  
ACGCAGCTATTGAGTCACTAGATCCATCAGGAGGTTTCAGAGA  
CTAAAGCACCTACTCCTGTAACAAATTCAGGAAGCAATAATC  
AAGAGAAAATAGCAACGCAAGGAAATTATACATTTTCACATA  
AAGTAGAAGTAAAAAATGAAGCTAAGGTAGCGAGTCCAACCTC  
AATTTACATTGGACAAAGGAGACAGAATTTTTTACGACCAAA  
TACTAACTATTGAAGGAAATCAGTGGTTATCTTATAAATCATT  
CAATGGTGTTTCGTCGTTTTGTTTTGCTAGGTAAAGCATCTTCA  
GTAGAAAAAACTGAAGATAAAGAAAAAGTGTCTCCTCAACCA  
CAAGCCCGTATTACTAAAACTGGTAGACTGACTATTTCTAACG  
AAACAACtACAGGTTTTGATATTTTAATTACGAATATTAAAGA  
TGATAACGGTATCGCTGCTGTTAAGGTACCGGTTTGGACTGAA  
CAAGGAGGGCAAGATGATATTAATGGTATACAGCTGTAACt  
ACTGGGGATGGCAACTACAAAGTAGCTGTATCATTTGCTGAC  
CATAAGAATGAGAAGGGTCTTTATAATATTCATTTATACTACC  
AAGAAGCTAGTGGGACACTTGTAGGTGTAACAGGAACtAAAG  
TGACAGTAGCTGGAACtAATTCTTCTCAAGAACCTATTGAAAA  
TGGTTTACCAAAGACTGGTGTTTATAATATTATCGGAAGTACT  
GAAGTAAAAAATGAAGCTAAAATATCAAGTCAGACCCAATTT  
ACTTTAGAAAAAGGTGACAAAATAAATTATGATCAAGTATTG  
ACAGCAGATGGTTACCAGTGGATTTCTTACAAATCTTATAGTG  
GTGTTTCGTCGCTATATTCTGTGAAAAAGCTAACTACAAGTAG  
TGAAAAAGCGAAAGATGAGGCGACTAAACCGACTAGTTATCC  
CAACTTACCTAAACAGGTACCTATACATTTACTAAAACTGTA  
GATGTGAAAAGTCAACCTAAAGTATCAAGTCCAGTGGAATTT  
AATTTTCAAAAGGGTGAAAAAATACATTATGATCAAGTGTTA  
GTAGTAGATGGTCATCAGTGGATTTTCATACAAGAGTTATTCCG  
GTATTCGTCGCTATATTGAAATTTAA

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MFMMKKGQVNDTKQSYSLRKYKFGLASVILGSFIMVTSPVFADQTTSVQVNN  
 QTGTSVDANNSSNETSASSVITSNNDQVQASDKVVNSQNTATKDITPLVETK  
 PMVEKTLPEQGNVYVYSKETEVKNTPSKSAPVAFYAKKGDKVFYDQVFNKDN  
 VKWISYKSFGGVRRYAAIESLDPSGGSETKAPTPVTNSGSNNQEKIATQGNYT  
 FSHKVEVKNEAKVASPTQFTLDKGDRIFYDQILTIEGNQWLSYKSFNGVRRFV  
 LLGKASSVEKTEDKEKVSPQPQARITKTGRLTISNETTTGFDILITNIKDDNGIA  
 AVKVPVWTEQGGQDDIKWYTAVTTGDGNYKVAVSFADHKNEKGLYNIHLY  
 YQEASGTLVGVTGKVTVAGTNSSQEPIENGLPKTGVYNIIGSTEVKNEAKISS  
 QTQFTLEKGDKINYDQVLTADGYQWISYKSYSGVRRYIPVKKLTSSEKAKDE  
 ATKPTSYPNLPKTGTYTFTKTVDVKSQPKVSSPVEFNFQKGEKIHVDQVLVVD  
 GHQWISYKSYSGIRRYIEI\*

## Sequence description

A) Length: 1642 bp - 547 aa (full length gene)

B) Sequence Characteristics:

Potential leader peptide sequence

Orf is preceded by a potential Shine-  
Dalgarno sequence.

ID-66

Clone 3-5

ATGATATTGAGACGTCGAACTATTGTTTTATGGCAACTGGGTATCGCCATT  
 TCTCTCATTCTTAGTATTCTAGCCTTAAATCTTTATTTCCATAGTACTCCCTT  
 GCAAACCAATGCAGCTTTACGGAACCTTGCTCCTTCATTAAACCATCTTTTT  
 GGGACAGATGGTTTAGGTAGGGATATGTTTGTGTCAGAACGATTAAAGGACT  
 TTATTTCTCTCTACAAGTCGGCTTATTAGGTGCCCTTATGGGGGTCATTCTG  
 GCGACAGTTTTTGGAGTGCTTGCAGGTTTAGGAAATAGCATTATTGATAAA  
 ATAATAGCATGGTTAGTTGATTGTTTATTGGTATGCCTCATTGATTTTTA  
 TGATTCTCATTCTTTTGTGTTGGGAAAGGTGCTCAAGGGGTCATCATTGC  
 AACGGCTGTTACACATTGGCCTTCTTTAGCAAGGCTTATCCGCAATGAAGT  
 CTATCATCTAAAGAATAAAGAATTTGTCCAACCTTTCTAAAAGTATGGGAAA  
 AACGCCTTATTATATTGTGAGGCATCATATCCTGCCTTTGATTGCTTCTCAA  
 ATTTTCATTGGTTTTATCCTCTTATTTCACATGTCATCCTACATGAAGCAT  
 CAATGACTTTCTTAGGATTTGGGCTCTCTGCCGAACAACCTTCGGTTGGTA  
 TCATTCTGTCAGAGGCAGCTAAGCATATCTCTCTTGGAATTGGTGGTTGG  
 TTATCTTTCCAGGACTTTATCTTATTTTGGTTGTCAATGCATTTGATACTAT  
 CGGAGAATCTTTAAAGAACTCTTTTACCCTCAAACCTGATCATTTTTAG

FIG. 1<sub>CONT'D</sub>

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MILRRRTIVLWQLGIAISLILSILALNLYFHSTPLQTNAALRNLAAPSLNHLFGTD  
 GLGRDMFVRTIKGLYFSLQVGLLGALMGVILATVFGVLAGLGNIIIDKIIAWL  
 VDLFIGMPHLIFMILISFVVVGKGAQGVIIATAVTHWPSLARLIRNEVYHLKNKE  
 FVQLSKSMGKTPYYIVRHHILPLIASQIFIGFILLFPHVILHEASMTFLGFGLSAE  
 QPSVGIIILSEAAKHISLGNWWLVIFPGLYLILVVNAFDTIGESLKKLFYPQTDHF  
 \*

#### Sequence description

A) Length: 822 bp - 274 aa (full length gene)

B) Sequence Characteristics:

Potential leader peptide sequence

Orf is preceded by a potential Shine-Dalgarno sequence.

ID-78

Clone 3-5b

ATGACAGAAACATTATTAAGCATTAAAGACCTCTCCATCACCTTCACTCAA  
 TACGGAAGATTTTTTAAAACCATTTCAATCAACACCGATAACAAGCGCTGA  
 ATTTAGAAATTAATAAAAGGTGAGTTATTAGCTATTATAGGTGCTAGTGGT  
 CGGGGAAGAGTTTATTAGCACATGCTATTATGGATATTCTTCCTAAAAATG  
 CATCTGTAACAGGAGATATGATTTATCGTGGTCAATCACTAAATTCTAAAC  
 GCATTAAACAGTTGCGAGGAAAAGATATTACGTTGATTCCACAATCAGTTA  
 ATTATTTAGATCCATCTATGAAAGTCAAACATCAGGTGCGCTTAGGTATCT  
 CAGAAAATTCAAAGGCTACTCAAGAAGGATTGTTTCAACAGTTTGGTTTAA  
 AAGAAAGTGATGGTGACTTGGATCCTTTCCAACCTTTCTGGCGGAATGCTCC  
 GACGTGTTTTGTTTACAACGTGTATTAGTGATAAGGTTTCTTTGATTATTGC  
 GGATGAGCCCACCCCTGGATTACATCCAGATGCTCTGCAAATGGTTTTAGA  
 CCAACTACGCTCCTTTGCAGATAAAGGAATAAGCGTTATATTTATCACTCA  
 TGATATTGTAGCAGCTAGTCAAATTGCTGATCGTATTACTATTTTTAAAGA  
 GGGAAAAGCTATTGAAACAGCTCCAGCTAGTTTCTTTAGCGGAAATGGAG  
 AGCAGTTACAAACAGAATTTGCTAGAAGTTTATGGCGCTCTCTCCACAGC  
 AAGAATTTTTGAAAGGAGTTACTCATGACCTTAGAGGCTAA

MTETLLSIKDLSITFTQYGRFLKPFQSTPIQALNLEIKKGELLAIIIGASGSGKSL  
 AHAIMDILPKNASVTGDMYRGQSLNSKRIKQLRGKDITLIPQSVNYLDPSMK  
 VKHQVRLGISENSKATQEGLFQQFGLKESDGDLDPFQLSGGMLRRVLFTTCIS  
 DKVSLIIADEPTPLHPDALQMVLDQLRSFADKGISVIFITHDIVAASQIADRITI  
 FKEGKAJETAPASFFSGNGEQLQTEFARSLWRSLPQQEFLKGVTHDLRG\*

**FIG. 1** CONT'D

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## Sequence description

A) Length: 804 bp - 268 aa (full length gene)

B) Sequence Characteristics:

No obvious leader peptide sequence

Orf is preceded by a potential Shine-Dalgarno sequence.

This gene was not isolated using the LEEP system. However in determining a full length gene sequence for ID-76, this gene was identified downstream and fully sequenced.

ID-79

Clone 3-5c

GTCCATCTGGGGTGGTTCCCGATTGGTATTTCTTCTCCGATAGGTACTTTGA  
 GTCAAGATATTACGTTAGCTGATCGTATTAAGCACCTTATTTTACCTGTTTT  
 CACGGTAAGTATTCTAGGCATTGCCAATGTAACCTTTCATACTAGAACTAA  
 AATGATGTCGGTACTTTCTAGTGAATATGTCTTATTTGCCAGAGCGCGTGG  
 GGAAACGGAATGGCAAATTTTTAAAAATCATTGTCTTAGAAATGCTATCGT  
 ACCAGCTATTACACTGCATTTTTCCTATTTTGGAGAATTGTTTGGAGGATCC  
 GTTCTTGCTGAGCAAGTTTTCTCATATCCAGGACTAGGGTCTACCCTAACT  
 GAAGCAGGACTTAAAAGTGATACACCGCTACTTCTAGCTATTGTGATGATA  
 GGGACATTATTTGTTTTTGCGGGCAATCTTATTGCGGATATTTTAAATAGC  
 ATAATCAATCCACAGTTAAGGAGAAAAGTATGA

VHLGWFPIGISSPIGTLSDITLADRIKHLILPVFTVSILGIANVTLHTRTKMMSV  
 LSSEYVLFARARGETEWQIFKNHCLRNAIVPAITLHFSYFGELFGGSVLAEQVF  
 SYPGLGSTLTEAGLKSDTPLLLAIVMIGTLFVFAGNLIADILNSIINPQLRRKV\*

## Sequence description

A) Length: 495 bp - 165 aa (partial gene sequence)

B) Sequence Characteristics:

N-terminus has yet to be determined.

This gene was not isolated using the LEEP system. However in determining a full length gene sequence for ID-76, this gene was identified upstream.

FIG. 1 CONT'D

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ID-80

Clone 2-17

TTGCGGACAATTACGTTCAAACACAATGAAACGCGATCGTCAAAAAGCGA  
AGGTAGGGCGGTAATGCTTAAAAGATTATTTACTGAAGATGGGGAATTGA  
CAAAGATTAGTCGTCGTTTCGTTTGGATGTTAGTGGTTATCTATTGTCTTAT  
TATTGTCAGGATGTGTTTTGGGCCTCAAATTATGATTGAGGGGGTATCAAC  
TCCGAATGTTTACGCGCTTCGGAAGAATTGTAGCTCTTTTAGTACCATTAA  
TTCTTTTCGTAGTTTAGATCAGCTAACTAGCTTTAAAGAGATTTTTTGGGT  
ATTGGTCAAAATGTAGTGAATATTTTACTGCTGTTTCCTCTCATTATAGGGT  
TACTATCCCTAAAGCCAAGTTTACGGAAATATAAAAGCGTTATATTACTTG  
CTTTCTTGATGTCTCTTTTCATAGAGTGTACTCAAGTTGTTTTAGATATTTT  
AATAGATGCTAATCGGGTTTTTCAAATCGACGATCTATGGACAAATACCTT  
AGGCGGTCCTTTCGCCCTATGGAGTTATCGAAACATAAAAGGTTGGCTTCT  
AACTATTAGAAAATGA

MRTITFKHNETRSSKSEGRAVMLKRLFTEDGELTKISRRFVWMLVVIYCLIIVR  
MCFGFPQIMIEGVSTPNVQRFGRIVALLVPFNSFRSLDQLTSFKEIFWVIGQNVV  
NILLFPLIIGLLSLKPSLRKYKSVILLAFMLSLFIECTQVVLIDILIDANRVFEIDD  
LWTNTLGGPFALWSYRNIKGWLLTIRK\*

## Sequence description

A) Length: 579 bp - 193 aa (full length gene)

B) Sequence Characteristics:

Possesses a potential leader peptide sequence

No obvious Shine-Dalgarno, but the 'TTG' codon  
may not be the actual translation start point.A methionine (ATG) that occurs ~22 codons  
downstream of the 'TTG' is preceded by a  
potential Shine-Dalgarno sequence and may  
represent the actual start codon.

ID 81

Clone 3-1

FIG. 1<sub>CONT'D</sub>

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TTGAAAAATTTAAATCGTTATGTAGTTGCGGTTTCTGGAGTCGTTTTACATT  
 TAATGCTAGGATCAACTTATGCTTGGAGTGTGTTTCGTAACCCAATTATCT  
 CAGAGACTGGTTGGGATATTTTCATCAGTTTCATTTCGCTTTTAGTTTGGCTAT  
 TTTTGTCTAGGAATGTCTGCAGCTTTTATGGGACACTTAGTAGAGCGTTTT  
 GGTCTAGGATAATGGGAATGATTTCTGCTATTTTATATGGAGCAGGGAAT  
 GTGTTAACAGGCTTAGCCATTGAACTCAGCAGTTATGGTTACTGTATGTT  
 GCATACGGTATTTTAGGAGGAATCGGACTTGGTTCAGGTTATATTACTCCA  
 GTATCGACTATTATTAAATGGTTTCCTGATAGGAGGGGACTAGCAACAGG  
 ATTCGCTATTATGGGATTTGGCTTTGCTTCTTTAGTAACAAGTCCGCTTGCA  
 CAATCCTTACTGATTAGGATTGGTGTGGGTAAAACGTTTTATATTTTGGGA  
 TTAGTATATTTTTTTTGTCTATGATGATTGCCTCACAATTTATTAAACAACCAC  
 CTCAGGAAAAAATAACTATTTTGACTCACGATGGTAAAAAGAATGCTATG  
 AATTCACAAATTATCACTGGATTAAAAGCAAACGTCGCTATAAAATCAAA  
 AACCTTTTACATCATTGTTGACCTTGTTTATTAATATTTTCGTGTGGCTTA  
 GGTTTAATATCAGCAGCTTCACCAATGGCACAAGATTTAGCAGGCTATTCC  
 GCAGAATCTGCAGCCTTATTAGTAGGGGTACTAGGGATATTTAACGGTTTT  
 GGACGTCTGTTATGGGCAAGTCTCTCTGACTACATTGGACGCCCGTTGACC  
 TTTATAATATTATTTATTGTGAACCTTATTATGACTTCTAGTTTATTTTTGTC  
 ATTCAATGCTATTGTATTTGCAATAGCGATGTCTATTTTAATGACTTGTTAT  
 GGTGCAGGTTTTTTCCTTATTACCTGCTTATCTAAGTGATATTTTGAACAA  
 AGGAATTAGCTACTTTACATGGTTATAGTTTAACAGCATGGGCAATAGCAG  
 GTCTGTTTGGGCCCCCTATTGTTATCAAAGACATATTCATGGGGAAATTCCT  
 ATCAATTGACATTAATGGTTTTTGGTTTTTTATTCTTATTCGGATTATTGTTA  
 TCTCTATATTTAAGAAAATTAACAATAAAGTTGTGTAG

LKNLNRYVVAVSGVVLHMLLGSTYAWSVFRNPIISETGWDISSVSFAFSLAIFC  
 LGMSAAFMGHLVERFGPRIMGMISAILYGAGNVLTGLAIETQQLWLLYVAYG  
 ILGGIGLGSYITPVSTIIKWFPDRRLATGFAIMGFGFASLVTSPLAQSLIRIG  
 VGKTFYILGLVYFFVMMIASQFIKQPPQEKITILTHDGKKNAMNSQIITGLKAN  
 VAIKSKTFYIIWLTFLFINISCGLGLISAASPMAQDLAGYSAESAALLVGVLGIFN  
 GFGRLLWASLSDYIGRPLTFIILFIVNFIMTSSLFLSFNAIVFAIAMSILMTCYGA  
 GFSLLPAYLSDFGTKELATLHGYSLTAWAIAGLFGPLLLSKTYSWGNSYQLTL  
 MVFGFLFLFGLLLSLYLRKLTTKVV\*

#### Sequence description:

- A] Length 1221 bp - 407 a.a (full length gene).
- B] TTG start codon with Shine-Dalgarno sequence upstream. Obvious signal peptide, with hydropathy plot exhibiting many possible membrane spanning regions, indicating protein to be transmembrane.

**FIG. 1** CONT'D

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ID-82

Clone 48

ATGGCAGATAAAAAACAGAACATTTAAACTTGTAGGTGCAGGATCTTCTAG  
CACACAAGAAAAAATTGAAAAGCCTGCTCTTTCGTTTATGCAAGATGCGTG  
GCGTCGCTTGAAAAAAAACAAATTAGCAGTAGTTTCACTCTATTTATTAGC  
TCTTTTACTTACTTTTTCGTTAGCCTCAAATTTATTTGTAAGTCAAGGAT  
GCTAATGGGTTTGATTTCGAAAAAAGTAACGACATATCGCAACTTACCACCT  
AAATTGAGTTCAAACCTTCCTTTTGGGAATGGTAGCATTAATCCATCA

MADKNRTFKLVGAGSSSTQEKIEKPALSFMQDAWRRLKKNKLAVVSLYLLA  
LLTFSLASNLFTQKDANGFDSKKVTTYRNLPPKLSSNLPFWNGSINPS

Sequence description:

A] Current length is 303 bp - 101 aa  
B] No obvious signal peptide but Shine  
Dalgarno sequence upstream of the ATG start  
codon. Not identified directly using the LEEP system but was found  
directly downstream of ID-34 described in WO 00/06736.

ID-83

Clone 98

ATGAAAATAGTAGTACCAGTAATGCCTCGCAGTCTTGAAGAGGCTCAAGA  
AATAGATTTATCAAAATTTGATAGTGTTGATATTATTGAATGGCGAGCTGA  
TGCCTTACCAAAGGATGACATTATTAATGTAGCTCCAGCTATTTTTGAGAA  
ATTCGCAGGTCATGAAATTATTTTTACTTTTCGTACAACGCGTGAAGGTGG  
TAATATTGTCTTATCTGATGCTGAGTATGTTGAGTTAATCCAGAAAATTAA  
TTCTATCTACAATCCAGATTATATTGATTTTGAGTATTTTTACATAAAGAA  
GTTTTTCAAGAAATGCTAGAATTTCCAAATTTAGTCCTGTCTTATCACAATT  
TTCAAGAGACACCGGAGAATATTATGGAGATATTTTCAGAATTAACAGCC  
CTAGCACCACGAGTTGTGAAAATCGCAGTAATGCCAAAGAATGAACAAGA

FIG. 1 CONT'D

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TGTCTTAGACGTTATGAATTACACTCGCGGTTTCAAGACTATTAATCCTGA  
TCAAGTTTATGCGACGGTATCTATGAGTAAAATTGGACGTATTTCTCGTTTT  
GCTGGTGATGTAAGTGGATCTAGTTGGACATTTGCATATTTAGATTCATCT  
ATCGCACCCGGACAAATTACTATTTTCAGAGATGAAGCGTGTCAAAGCATT  
GCTTGACGCTGACTGA

MKIVVPVMPRSLEEAEIDLSKFDSVDIIEWRADALPKDDIINVAPAIFEK FAG  
HEIIFTFRTTREGGNIVLSDAEYVELIQKINSIYNPDYIDFEYFSHKEVFQEMLEF  
PNLVLSYHNFQETPENIMEIFSELTALAPRVVKIAVMPKNEQDVLDMNYTRG  
FKTINPDQVYATVSMKIGRISRFAGDVTGSSWTFAYLDSSIAPGQITISEMKRV  
KALLDAD\*

Sequence description:

- A] Length 678 bp, 225 aa (full length gene)
- B] No obvious signal peptide, but there is a Shine Dalgarno immediately upstream of ORF.

ID-84

Clone RS-52

ATGAAAGACTTATTTGCAACAACAGAAGCATCATCAAGGAAACAGGAACA  
AGATAGAATTGTCAATTACATAAAACAACATGTTGAGTTAACAAATGGTA  
ATCAAATAAAAAAAATTGAGTTTATCGACTTTCAAAAAAATGAGATGACA  
GGTACATGGGGAATTTCTACTAAAATTAATGAACAATTTTCGATTAGTTTT  
TCTGAAGATAGAATTGGTGGTAAACTTAGAGCATTAGGATATCAACCGAA  
TGAAATAGGTTTTTTCAAAGGACATCAATAGTAATAATCAAATGTTAATGA  
TATTGAAGTGATTTATATGAAGAAAGAATAG

MKDLFATTEASSRKQEQRIVNYIKQHVELTNGNQIKKIEFIDFQKNEMTGTW  
GISTKINEQFSISFSEDRIGGKLRLALGYQPNEIGFSKDINSNNQNVNDIEVIYMK  
KE\*

Sequence description:

- A] length: 333 bp - 111 aa (partial sequence)
- B] No obvious Shine Dalgarno sequence upstream of the ATG start codon, and no obvious signal peptide within the protein.

FIG. 1<sub>CONT'D</sub>

9 / 110

ID-85

Clone RS-53

ATGAAAAAACGTATATGGTATTTGATAATAATAATCACAGTAATTTTAGGA  
GGACTAGCCATGAAAAACTTATTTGCAACAACAGAAGCATCATCAAGGAA  
ACAGGAACAAGATAGAATTGTCAATTACATAAAACAACATGTTGAGTTAA  
CAAATGGTAATCAAATAAAAAAAATTGAGTTTATCGACTTTCAAAAAAAT  
GAGATGACAGGTACATGGGGAATTTCTACTAAAATTAATGAACAATTTTCG  
ATTAGTTTTTCTGAAGATAGAATTGGTGGTAACTTAGAGCATTAGGATAT  
CAACCGAATGAAATAGGTTTTTCAAAGGACATCAATAGTAATAATCA

MKKRIWYLIHITVILGGLAMKNLFATTEASSRKQEQRIVNYIKQHVELTNGN  
QIKKIEFIDFQKNEMTGTWGIS TKINEQFSISFSEDRIGGKLRALGYQPNEIGFSK  
DINSNNQ

Sequence description:

- A] Length: 351 bp - 117 aa (Partial sequence)
- B] Obvious signal peptide and Shine Dalgarno  
sequence upstream of the ATG start codon.

ID-86

Clone ID-74

ATGTCAAATCAATATGATTATATCGTTATTGGTGGAGGTAGT  
GCAGGCAGTGGTACCGCTAATAGGGCAGCCATGTATGGAGC  
AAAAGTCCTGTTAATTGAAGGTGGACAAGTAGGTGGAACCTG  
TGTTAACTTAGGTTGTGTACCTAAGAAAATCATGTGGTATGG  
TGCACAAGTTTCTGAGACACTCCATAAGTATAGTTCAGGTTA  
TGGTTTTGAAGCCAATAATCTTAGTTTTGATTTTACTACTCTA  
AAAGCTAATCGCGATGCTTACGTGCAGCGGTCTAGACAGTCG  
TATGCCGCTAATTTTGAGCGTAATGGGGTCGAAAAGATTGAT  
GGATTTGCTCGTTTTATTGATAACCATACTATTGAAGTGAATG  
GTCAGCAATATAAAGCTCCTCACATTACTATTGCAACAGGTG

FIG. 1<sub>CONT'D</sub>

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GACACCCTCTTTACCCTGATATTATTGGAAGTGAACCTTGGTG  
AGACTTCTGATGATTTTTTTTGGATGGGAGACCTTACCAAATTC  
TATATTGATTGTTGGGGCGGGCTATATCGCGGCAGAACTTGC  
TGGAGTGGTTAATGAATTAGGCGTTGAAACCCATCTTGCATT  
TAGAAAAGACCATATTCTACGCGGATTTGATGACATGGTAAC  
AAGTGAGGTTATGGCTGAAATGGAGAAATCAGGTATCTCTTT  
ACATGCTAACCATGTACCTAAATCTCTTAAACGCGATGAAGG  
TGGCAAGTTGATTTTTTGAAGCTGAAAATGGGAAAACGCTTGT  
CGTTGATCGTGTAATATGGGCTATCGGCCGTGGACCAAATGT  
AGACATGGGACTTGAAAATACCGATATTGTTTTAAATGATAA  
AGATTATATCAAAACAGATGAATTTGAGAATACTTCTGTAGA  
TGGCGTGTATGCTATTGGAGATGTTAATGGGAAAATTGCCTT  
GACACCGGTAGCAATTGCAGCAGGTCGTCGCTTATCAGAAAG  
ACTTTTTAATCATAAAGATAACGAAAAATTAGATTACCATAA  
TGTACCTTCAGTTATTTTTACTCACCTGTAATTGGGACGGTA  
GGACTTTCAGAAGCAGCAGCTATCGAGCAATTTGGAAAAGAT  
AATATCAAAGTCTATACATCAACTTTTACCTCTATGTATACGG  
CTGTTACCAGTAATCGCCAAGCAGTTAAGATGAAGCTCATAA  
CCCTAGGAAAAGAGGAAAAAGTTATTGGGCTTCATGGTGTG  
GTTATGGTATTGATGAAATGATTCAAGGTTTTTCAGTTGCTAT  
CAAAATGGGGGCTACTAAAGCAGACTTTGATGATACTGTTGC  
TATTCACCCAACCTGGATCTGAGGAATTTGTTACAATGCGCTA  
A

MSNQYDYIVIGGGSAGSGTANRAAMYGAKVLLIEGGQVGGTC  
VNLGCVPKKIMWYGAQVSETLHKYSSGYGFEANNLSFDFTTLK  
ANRDAYVQRSRQSYAANFERNGVEKIDGFARFIDNHTIEVNGQ  
QYKAPHITIAATGGHPLYPDIIGSELGETSDDFFGWETLPNSILIVG  
AGYIAAELAGVVNELGVETHLAFRKDHLRGFDDMVTSEVMAE  
MEKSGISLHANHVPKSLKRDEGGKLIFEANGKTLVVDRVIWAI  
GRGPNVDMGLENTDIVLNDKDYIKTDEFENTSVDG VYAIGDVN  
GKIALTPVAIAAGRRLSERLFNHKDNEKLDYHNVPSVIFTHPVIG  
TVGLSEAAAIEQFGKDNKVYTSTFTSMYTAVT SNRQAVKMKLI  
TLGKEEKVIGLHGVGYGIDEMIQGFSAIKMGATKADFDDTVAI  
HPTGSEEFVTMR\*

ID-87

FIG. 1<sub>CONT'D</sub>

SUBSTITUTE SHEET (RULE 26)

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Clone RS-55

ATGACAAAAAACATCTTAAAACGCTTGCCTTGGCACTTACTACAGTATCA  
GTAGTGACATACAGCCAGGAGGTATATGGATTAGAAAAGAGAGGAATCGGT  
CAAACAAGAACAAACCCAGTCAGCTTCAGAAGATGATTGGTTCGAAGAAG  
ATAATGAGAGGAAAACAAATGTTTCTAAAGAGAATTCTACTGTTGATGAA  
ACAGTTAGTGATTTATTTTCTGATGGAAATAGTAATAACTCTAGTTCTAAA  
ACCGAGTCAGTGGTAAGTGACCCTAAACAAGTCCCCAAAGCAAAACCAGA  
GGTTACACAAGAAGCAAGCAATTCTAGTAATGATGCTAGCAAAGTAGAAG  
TACCAAAACAGGATACAGCTTCAAAAAAGGAACTCTAGAAACATCAACT  
TGGGAGGCCAAAAGATTTTCGTAAGTCTAGAGGGGATACTTTAGTAGGTTTTTCA  
AAATCTGGAATTAATAAGTTATCTCAAACATCACACTTGGTTTTTACCAAGT  
CATGCAGCAGATGGAAGTCAATTGACACAAGTAGCTAGCTTTGCTTTTACT  
CCAGATAAAAAGACGGCCATTGCAGAATATACAAGTAGGCTAGGAGAAA  
ATGGGAAACCGAGTCGTTTAGATATTGATCAGAAGGAAATTATTGATGAG  
GGAGAAATATTTAATGCTTACCAGTTGACTAAGCTTACTATTCCAAATGGT  
TATAAGTCTATTGGTCAAGATGCTTTTGTGGACAATAAGAATATTGCTGAG  
GTTAACCTTCCTGAGAGTCTCGAGACTATTTTCAGACTATGCTTTTGCTCACA  
TGTCTTTAAAACAAGTAAAGTTACCAGATAACCTAAAGGTCATTGGAGAA  
TTAGCTTTTTTTGATAATCAGATTGGTGGTAAGCTTTACTTGCCACGTCCT  
TGATAAAATTAGCAGAACGCGCTTTCAAATCTAATCGTATTCAAACAGTTG  
AATTTTTGGGAAGTAAGCTTAAGGTTATAGGAGAAGCAAGTTTTCAAGAT  
AATAATCTGAGGAATGTTATGCTTCCGGATGGACTTGAAAAAATAGAATC  
AGAAGCTTTTACAGGAAATCCAGGAGATGAACATTACAACAATCAGGTTG  
TATTGCGCACAAGGACAGGCCAAAATCCACATCAACTTGCGACTGAGAAT  
ACTTACGTCAATCCGGACAAATCATTGTGGCGTGCAACACCTGATATGGAT  
TATACCAATGGTTAGAGGAAGATTTTACCTATCAAAAAAATAGTGTTACA  
GGTTTTTCAAATAAAGGCTTACAAAAGGTAAGACGTAATAAAAACTTAGA  
AATTCCAAAACAACACAATGGTATTACTATTACTGAAATTGGTGATAACGC  
TTTTCGCAATGTTGATTTTCAAAGTAAAACCTTACGTAAATATGATTTGGA  
AGAAATAAAGCTCCCCTCAACTATTCGGAAAATAGGTGCTTTTGCTTTTCA  
ATCTAATAACTTGAAATCCTTTGAAGCAAGTGAAGATTTAGAAGAGATTA  
AAGAGGGAGCCTTTATGAATAATCGTATTGGAAGTCTAGACTTGAAAGAC  
AACTTATCAAAATAGGTGATGCTGCTTTCCATATTAATCATATTTATGCC  
ATTGTTCTTCCAGAATCTGTACAAGAAATAGGACGTTTCAGCTTTTCGACAA  
AATGGTGCGCTTCACCTTATGTTTATCGGAAATAAGGTTAAACAATTGGT  
GAAATGGCTTTTTTATCCAATAAACTGGAAAGTGTAATCTCTCTGAGCAA  
AAACAATTAAAGACAATTGAGGTCCAAGCTTTTTTCGGATAATGCCCTTAGT  
GAAGTAGTCTTACCGCCAAATTTACAGACTATTCGTGAAGAGGCTTTCAA  
AGGAATCATTTGAAAGAAGTGAAGGGTTCATCTACATTATCTCAGATTACT  
TTAATGCTTTTGATCAAAATGATGGGGACAAACGCTTTGGTAAGAAAGTG  
GTTGTTAGGACACATAATAATTCTCATATGTTAGCAGATGGTGAGCGTTTT  
ATCATTGATCCAGATAAGCTATCTTCTACAATGGTAGACCTTGAAAAGGTT

FIG. 1 CONT'D

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TTAAAAATAATCGAAGGTTTAGATTACTCTACATTACGTCAGACTACTCAA  
 ACTCAGTTTAGAGAAATGACTACTGCAGGTAAAGCGTTGTTATCAAAATCT  
 AACCTCCGACAAGGAGAAAAACAAAAATTCCTTCAAGAAGCACAATTTTT  
 CCTTGGTCGCGTTGATTTGGATAAAGCCATAGCTAAAGCTGAGAAGGCTTT  
 AGTGACCAAGAAGGCAACAAAGAATGGTCATTTGCTTGAGAGGAGTATTA  
 ACAAAGCGGTATTAGCTTATAATAATAGTGCTATTAAAAAAGCTAATGTTA  
 AGCGCTTGGAAAAAGAGTTAGACTTGCTGACAGATTTAGTCGAGGGGAAAA  
 GGACCATTAGCGCAAGCTACAATGGTACAAGGAGTTTATTTATTAAAGAC  
 GCCTTTACCATTGCCAGAATATTATATCGGATTGAACGTTTATTTTGACAA  
 GTCTGGAAAATTGATTTATGCACTTGATATGAGTGATACTATTGGCGAGGG  
 AAAAAAGATGCATATGGTAATCCTATATTAAATGTTGACGAGGATAATG  
 AAGGTTATCATACCTTGGCAGTTGCCACTTTAGCTGATTATGAAGGTCTTT  
 ATATTAAAGATATTTTAAATAGTTCCCTTGATAAGATTAAAGCAATACGCC  
 AGATTCCTTTGGCAAAATATCATAGATTAGGAATTTTCCAAGCTATCCGAA  
 ATGCAGCGGCAGAAGCAGACCGATTGCTTCCTAAGACACCTAAGGGGTAC  
 CTAAATGAAGTCCCAAATTATCGTAAAAAACAAATGGAGAAAAATTAAA  
 ACCAGTTGATTATAAAACGCCGATTTTAAATAAGGCTTTACCTAATGAAAA  
 GGTAGACGGTGATAGAGCGGCTAAAGGTCATAATATAAATGCGGAGACTA  
 ATAATTCTGTAGCTGTAAACACCAATAAGGTCCGAGCAGCAATTACATAAGT  
 CACAGTCTGATGTAAATTTACCTCAAACAAGTTCTAAAAATAATTTTATAT  
 ACGAGATTCTAGGATACGTTAGTTTATGTTTGCTTTTCCTAGTAACTGCTGG  
 GAAAAAAGGAAAACGAGCAAGAAAAATAA

MTKKHLKTLALALTTVSVVTYSQEVYGLEREESVKQEQTQSASEDDWFEEDN  
 ERKTNVSKENSTVDETVDLFSDBGNSNNSSSKTESVVSDPKQVPKAKPEVTQE  
 ASNSSNDASKVEVPKQDTASKKETLETSTWEAKDFVTRGDTLVGFSKSGINKL  
 SQTSHLVLP SHAADGTQLTQVASFATPDKKTAIAEYTSRLGENGKPSRLDIDQ  
 KEIIDEGEIFNAYQLTKLTIPNGYK SIGQDAFVDNKNIAEVNLPESLETISDYAF  
 AHMSLKQVKLPDNLK VIGELAFFDNQIGGKLYLPRHLIKLAERAFKSNRIQTV  
 EFLGSKLVIGEASFQDNNLRNVMLPDGLEKIESEAF TGNPGDEHYNNQVVL R  
 TRTGQNP HQ LATENTYVNPDKSLWRATPDMDYTKWLEEDFTYQKNSVTGFS  
 NKGLQKVR RNKNLEIPKQHNGITITEIGDNAFRNVDFQSKTLRKYDLEEIKLPS  
 TIRKIGAF AFQSNNLKSFEASEDLEEIKEGAFMNNRIGTLDLKD KLIKIGDAAFH  
 INHIYAIVLPESVQEIGRSAFRQNGALHLMFIGNKVKTIGEMAFLSNKLESVNL  
 SEQKQLKTIEVQAFSDNALSEVVLPPNLQTIREEAFKRNHLKEVKGSSTLSQITF  
 NAFDQNDGD KRF GKKVVRTHNNSHMLADGERFIIDPDKLSSTMDLEKVL  
 KIIEGLDYSTLRQTTQTQFREMTTAGKALLSKSNLRQGEKQKFLQEAQFFLGR  
 VDLDKAIKAEKALVTKKATKNHLLERSINKAVLAYNNSAIKKANVKRLEK  
 ELDLLTDLVEGKGPLAQATMVQGVYLLKTPPLPEYYIGLNVYFDKSGKLIYA  
 LDMSDTIGEGQKDAYGNPILNVDEDNEGYHTLAVATLADYEGLYIKDILNSSL  
 DKIKAIRQIPLAKYHRLGIFQAIRNAAAEADRLLPKTPKGYLNEVPNYRKKQM  
 EKNLKPVDYKTPIFNKALPNEKVDGDRAAKGHNINAETNNSVAVTPIRSEQQL  
 HKSQSDVNLPQTSSKNNFIYEILGYVSLCLLFLVTAGKKGKRARK\*

FIG. 1 CONT'D

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## Sequence description:

- A] Length 3168 bp - 1056 aa (Partial sequence)
- B] Obvious signal peptide with Shine Dalgarno sequence upstream of the ATG start codon.

ID-88

Clone RS-56

GCAGGATACATCATGCACAAGCACGAGGCTATCGTGTCATGCTGGGGTCA  
ACCCAGGAAGACATGTCCGCACAAGCTGAAGATTTCTTTACAGTCTGTACA  
CAATAAAGAGACGGGTAAGAGCGCTTTTAATGACAAAGAACGACTAGCAA  
TT

AGYIMHKHEAIVSCWGQPRKTCRHKLKISLQSVHNKETGKSAFNDKERLAI

## Sequence description:

- A] Length:153 bp - 51 aa (partial sequence)
- B] No signal peptide visible, insufficient sequence data to determine the presence of a Shine Dalgarno sequence.

ID-89

Clone RS-58

GTGTCATTTATGCAAAGAAAATCCTATTTAAAATCCATGAGTGTTCTTACT  
TTAACAGCTTGTCTTATATCAGGATATGTGGTTAAAGATATTGCTATGTTA  
CATGCAGTATCTGCCAGTGAGAAGAAAGCAAATAATGTCAGTCCGAGAGA  
AAATCTCTACAGGGCTGTCAATGATAATTGGCTAGCCAATACAAAACCTCA  
AACAAAGGGCAGACTAGTGTTAATAGTTTTTCAGAAATTGAGGATAAATTA  
AAGCAACTGTTAGTGTCTGATATGGCTAAAATGGCCTCAGGAAAGATTGA

FIG. 1<sub>CONT'D</sub>

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AACAACCAATGATGAACAGAAAAAATGGTTGCATACTATAAACAAGGTA  
TGGACTTTAAAAACAAGAGATAAAAAATGGTCTCAAACCTCTAAAACCAGTT  
TTACAAAAAATTGAAGCAGTCTCTTCAATGAAAGACTTTCAAAGTTTGGCC  
CATGATTTTGTGATGAGTGGTTTTGTTTTACCATTGTTGGTTGACTGTGGAAA  
CCAATGCTCGAGATAATAGCCAAAAGCAATTGGTGCTTCGTCAAGCACCC  
GCATTACTTGAATCACCTGACCAATATAAGAAGGGCAATAAAGAAGGTGA  
GGCTAAATTATCAGCTTACCGTACTTCAGCAATGGCTTTGCTTAAACAAGC  
TGGAAAAAGTAACATTGAAGATAGAAAACTAGTTAAACAAGCTATAGCAT  
TTGATAGACTCTTATCAGAAAAAACGCAAGTTGATCAAAGTAAAAATCACA  
GCTGAAAGTGAGACAGCTGCGGGGCGATATAACCCTGAAAGTATGGAAAC  
GGTTCACAATTACGCCAAGGAATTTGACTTTAAAGAATTGATTGAAAAACT  
AGTTGGGCCAACGAATAAGGCAGTCAATGTAGAAGATAAAACTTATTTTA  
AACAGGTTAATGATGTTATAAATAGTAAACAATTAGCCAATATGAAAGCA  
TGGATGATGATTTCTATGCTAGTTGATCAATCAGATTTTCTAGGAGAACAA  
AATCGTCAAGCAGCGAGTGCTTTTAAGAATGTTGCGTCTGGTTTGACTCAG  
ATTGAATCGAAAGAAAAAATGCTTACACCCAATTAG

MSFMQRKSYLKSM SVLTLTACLISGYVVKDIAMLHAVSASEKKANNVSPREN  
LYRAVNDNWLANTKLKQGQTSVNSFSEIEDKLKQLLVSDMAKMASGKIETTN  
DEQKKMVAYYKQGMDFKTRDKNGLKPLKPV LQKLEAVSSMKDFQSLAHDF  
VMSGFVLPFGLTVETNARDNSQKQLVLRQAPALLES PDQYKKGNKEGEAKLS  
AYRTSAMALLKQAGKSNIEDRKLVKQAI AFDRLLESEKTQVDQSKITAESETAA  
GRYNPESMETVHNYAKEFDKELIEKLVGPTNKA VNVEDKTYFKQVNDVINS  
KQLANMKAWMMISMLVDQSDFLGEQNRQAASAFKNVASGLTQIESKEKMLT  
PN\*

Sequence description:

- A] Length: 1095 bp - 365 aa (full length gene)
- B] an GTG (possible ATG start codon located 7 bp further downstream) start codon with an obvious signal peptide. Shine Dalgarno sequence present upstream of the ORF.

ID-90

Clone RS-59

FIG. 1<sub>CONT'D</sub>

15 / 110

ATGGAAATGCCTAAAAGAAATGAATTACTCAATAAAGAAATTAAAATGAG  
TATTGATAAACTTAGATATAAAGAACCAGAGAGTGAACATGACAAGCGAC  
CTACTTTTTTATTTGGTAGTACTTATACTTGTTACTGTAGCAGTTATATTGTC  
GTTATTTAAATATTTTTTATAG

MEMPKRNELLNKEIKMSIDKLRYKEPESEHDKRPTFYLVVLILVTVAVILSLFK  
YFL\*

Sequence description:

- A] Length: 174 bp - 58 aa(full length gene)
- B] No obvious signal peptide, but Shine  
Dalgarno sequence is present upstream of ATG  
start codon.

ID-91

Clone RS-62 (partial sequence)

ATGCAGGTATTTTTTAAATATTGTCAATAAATTCTTTGATCCAGTTATTCATA  
TGGGTTTCGGGAGTTGTGATGCTAATTGTCATGACAGGTTTAGCCATGATAT  
TTGGAGTGAAGTTTTCTAAAGCACTTGAAGGTGGTAT

MQVFLNIVNKFFDPVIHMGSGVVMLIVMTGLAMIFGVKFSKALEGG

Sequence description:

- A] Length:141 bp - 41 aa (partial sequence
- B] Shine Dalgarno sequence present upstream of  
ATG start codon with a possible signal peptide  
present

ID-92

FIG. 1<sub>CONT'D</sub>

16 / 110

Clone RS-69 (partial sequence)

ATGAAAAAGAAAACATTCAGTGCTTATAACTTTTTTAACGGCTCTTATCCTT  
TGTCTTTTGACAGTGCTTTTTATCTTTCCATTTTATTGGATTATGACAGGAG  
CTTTTAA

MKKKTF SAYNFL TALILCLLTVLFIFPFYWIMTGAF

Sequence description:

- A] Length: 110 bp -36 aa (Partial sequence)
- B] Possible signal peptide with Shine Dalgarno sequence directly upstream of the ATG start codon.

ID-93

Clone RS-70

ATGACTGAGAACTGGTTACATACTAAAGATGGTTCAGATATTTATTATCGT  
GTCGTTGGTCAAGGTCAACCGATTGTTTTTTTACATGGCAATAGCTTAAGT  
AGTCGCTATTTTGATAAGCAAATAGCATATTTTTCTAAGTATTACCAAGTT  
ATTGTTATGGATAGTAGAGGGCATGGCAAAAGTCATGCAAAGCTAAATAC  
CATTAGTTTCAGGCAAATAGCAGTTGACTTAAAGGATATCTTAGTTCATTT  
AGAGATTGATAAAGTTATATTGGTAGGCCATAGCGATGGTGCTAATTTAGC  
TTTAGTTTTTCAAACGATGTTTCCAGATATGGTTAGAGGGCTTTTGCTTAAT  
TCAGGGAACCTGACTATTCATGGTCAGCGATGGTGGGATATTCTTTTAGTA  
AGGATTGCCTATAAATTCCTTCACTATTTAGGGAACTCTTCCGTATATG  
AGGCAAAAAGCTCAAGTTATTTGCTTATGTTGGAGGATTTGAAGATTAGT  
CCAGCTGATTACAGCATGTGTCAACTCCTGTAATGGTTTTGGTTGGAAAT  
AAGGACATAATTAAGTTAAATCATTCTAAGAACTTGCTTCTTATTTTCCA  
AGGGGGGAGTTTTATTCTTTAGTTGGCTTTGGGCATCACATTATTAAGCAA  
GATTCCCATGTTTTTAATATTATTGCAAAAAAGTTTATCAACGATACGTTG  
AAAGGAGAAATTGTTGAAAAAGCTAATTGA

MTENWLHTKDGSDIYYRVVGQGQPIVFLHGNSLSSRYFDKQIAYFSKYYQVIV  
MDSRGHGKSHAKLNTISFRQIAVDLKDILVHLEIDKVILVGHSDGANLALVFQ

FIG. 1<sub>CONT'D</sub>

17 / 110

TMFPDMVRGLLLNSGNLTIHGQRWWDILLVRIAYKFLHYLGKLFYPYMRQKA  
QVISLMLEDLKISPADLQHVSTPVMVLVGNKDIKLNHKKLASYPFRGEFYSL  
VGFGHHIIKQDSHVFNIIAKKFINDTLKGEIVEKAN\*

Sequence description:

A] Length: 744 bp - 248 aa (full length gene)  
B] No obvious signal peptide, but Shine  
Dalgarno sequence upstream of the ATG start  
codon.

ID-94

Clone RS-71

ATGGTAGCAAAAGAGTTAGGTAAAAATAGCTTTACTATCCCAACTATTTGT  
TCTAATTGCTCCGCAGGTACTGCCATTGCAGTTGTATATAATGATGACCAT  
TCTTCTTAAGATACGGCTATCCCGAGTCTCCACTTCATATTTTTTATCAATA  
CACGGATCATTGCACAGGCACCAAGCAAATATTTTTGGGCTGGTATTGGGG  
ACGGTATTTCAAAAGCCCCTGAAGTAGAACGTGCTACCTTAGAGGCTAAG  
ACCAATAAACTACCACATACTGCAGTGTTAGGACAAGCAGTCGCTCTGTCT  
TCAAAGGAAGCTTTTTATCAATTTGGTGAACAAGGTCTAAAAGACGTTGAA  
GCTAATTTAGCTTCGCGTGCAGTTGAAGAAATTGCGCTTGATATCTTA

MVAKELGKNSFTIPTICSNCSAGTAIAVVYNDHDSFLRYGYSPESPLHIFINTRIIA  
QAPSKYFWAGIGDGISKAPEVERATLEAKTNKLPHTAVLGQAVALSSEAFY  
QFGEQGLKDVEANLASRAVEEIALDIL

Sequence description:

A] Length: 405 bp - 135 aa (Partial sequence)  
B] No obvious Shine Dalgarno sequence upstream  
of the ATG start codon, probable signal  
peptide present at the N-terminus.

ID-95

**FIG. 1** CONT'D

18 / 110

Clone RS-73

TTGAGGGGAACTTACTGGAAAATTTCAAGCGATTGCGATAAAATAAATCTT  
GCAGAGTTTTCTAGAGAAAGGAGGTCAGATTTATTGGAGTGGCAAGATCT  
AGCGCAGTTACCTGTATCTATTTTTAAAGACTATGTTACAGATGCTCAAGA  
CGCGGAAAAACCTTTTATATGGACAGAAGTATTTTTAAGGGAGATTAATCG  
CTCAAATCAAGAAATTATTTTGCATATTTGGCCGATGACTAAGACAGTCAT  
TCTGGGGATGTTAGATCGAGAATTACCACATTTAGAATTAGCTAAAAAAG  
AAATCATCAGTCGTGGTTATGAACCAGTTGTTTCGGAATTTTGGAGGTCTCG  
CAGTTGTAGCTGATGAAGGAATTTTAAATTTTTCATTGGTTATTCCAGATGT  
TTTTGAGAGAAAATTGTCTATCTCAGATGGGTATCTTATAATGGTCGATTTT  
ATTAGAAGTATATTTTCGGATTTTATCAACCTATTGAGCACTTTGAAGTA  
GAGACCTCCTATTGTCCTGGTAAGTTTGATCTTAGTATAAATGGCAAAAAA  
TTTGCTGGCTTGGCTCAGCGCCGTATAAAGAATGGTATTGCGGTATCAATT  
TACCTTAGCGTTTGTGGCGATCAAAAAGGGCGGAGTCAAATGATTTTCAGAT  
TTTTATAAGATTGGTCTAGGTGATACGGGTAGTCCAATTGCTTATCCAAAT  
GTAGATCCTGAAATTATGGCTAATCTATCTGATCTATTAGATTGTCCTATG  
ACAGTAGAAGATGTTATTGATCGTATGTTGATTAGCCTTAAACAAGTAGGT  
TTAATGATCGTTTACTGATGATTAGACCCGATTTAGTTGCAGAGTTTGAT  
AGATTTTCAGGCTAAGTCTATGGCTAATAAGGGGATGGTGAGCAGAGATGA  
ATAA

MRETYWKISSDCDKINLAEFSRERRSDLLEWQDLAQLPVSIFKDYVTDAQDAE  
KPFIWTEVFLREINRSNQEIILHIWPMKTKTVILGMLDRELPHLELAKKEIISRGYE  
PVVRNFGGLAVVADEGILNFSLVIPDVFERKLSISDGYLIMVDFIRSIFSDFYQPI  
EHFEVETSYCPGKFDLSINGKKFAGLAQRRIKNGIAVSIYLSVCGDQKGRSQMI  
SDFYKIGLGDTSPIAYPNVDPEIMANLSDLLDCPMTVEDVIDRMLISLKQVGF  
NDRLLMIRPDLVAEFDRFQAKSMANKGMVSRDE\*

Sequence description:

- A) Length: 921 bp -307 aa (Full-length gene sequence)
- B) No obvious Shine Dalgarno sequence upstream of the TTG start codon or signal peptide visible. Actual start point may be a further 85 bp downstream (TTG). This start point is preceded by a typical Shine-Dalgarno sequence.

FIG. 1<sup>CONT'D</sup>

19 / 110

ID-96

Clone RS-74

TTGGAAGGTTTACTTATTGCATTGATTCCCATGTTTGCCTGGGAAAGTATT  
GGATTTGTTAGTAATAAAATTGGAGGGCGTCCAAATCAACAAACATTTGG  
AATGACTTTAGGAGCATTGCTATTTGCGATTATCGTATGGTTATTTAAACA  
GCCAGAGATGACTGCCTCATTGTGGATTTTGGTATCTTAGGTGGTATCCT  
ATGGTCAGTCGGCCAAAATGGTCAATTTCAAGCAATGAAATATATGGGAG  
TCTCTGTTGCTAATCCACTGTCAAGTGGTGCACAATTAGTAGGTGGAAGCC  
TAGTTGGTGCTTTAGTCTTTCATGAATGGACTAAGCCAATCCAATTTATTTT  
AGGATTGACAGCGTTGACATTATTAGTTATCGGCTTCTATTTCTCAAGTAA  
ACGTGATGTTTCAGAACAAGCTTTGGCAACACATCAAGAGTTTTCAAAAG  
GATTTGCTACAATTGCTTATTCAACTGTAGGTTACATCTCGTACGCAGTTTT  
ATTTAACAACATTATGAAGTTCGACGCTATGGCCGTCATTTTACCCATGGC  
TGTTGGAATGTGTCTAGGTGCAATTTGTTTCATGAAGTTTCGTGTAACTTT  
GAGGCTGTTGTTGTTAAAAATATGATTACAGGTCTCATGTGGGGCGTTGGT  
AATGTCTTCATGTTATTGGCAGCAGCTAAAGCAGGGCTAGCAATTGCTTTT  
AGTTTTTCTCAACTTGGAGTAATTATCTCTATTATTGGTGGTATTTTATTTT  
AGGTGAGACAAAAACGAAGAAAGAGCAGAAATGGGTTGTCATGGGTATC  
CTTGTTTTGTTATGGGTGCTATATTACTTGGTATTGTTAAATCTTATTAA

MEGLLIALIPMFAWESIGFVSNKIGGRPNQQTFGMTLGALLFAIIVWLFKQPEM  
TASLWIFGILGGILWSVGQNGQFQAMKYMGVSVANPLSSGAQLVGGSLVGAL  
VFHEWTKPIQFILGLTALTLLVIGFYFSSKRDVSEQALATHQEFSKGFATIAYST  
VGYSISYAVLFNNIMKFDAMAVILPMAVGMCLGAICFMKFRVNFEAVVVKNMI  
TGLMWGVGNVFMLLAAAKAGLAIAFSFSQLGVIISIIGGILFLGETKTKKEQK  
WVVMGILCFVMGAILLGIVKSY\*

Sequence description:

- A] Length: 867 bp - 289 aa (full-length gene)
- B] Possible Shine Dalgarno sequence upstream of  
GTG start codon, no obvious signal peptide  
present.

ID-97

FIG. 1 CONT'D

20 / 110

Clone RS-75

ATGACAACCTTACTACGAAGCTATAAACTGGAACGAAATTGAAGATGTTAT  
 TGATAAATCAACTTGGGAAAACTAACCGAACAATTTTGGCTCGATACAC  
 GTATCCCTTTATCAAATGACTTAGACGATTGGCGCAAACCTTTCCGCTCAAG  
 AAAAAGATCTTGTTGGCAAGGTTTTTGGAGGCTTAACCCTACTTGATACCA  
 TGCAATCAGAACTGGTGTGAAGCTATTCGTGCCGATGTTTCGCACGCCTC  
 ACGAAGAAGCTGTCTTAAACAATATTCAATTCATGGAATCTGTTACGCTA  
 AATCTTATTCTTCAATTTTCTCAACTTTAAATACTAAATCAGAAATTGAAG  
 AAATTTTCGAGTGGACTAATAATAATGAGTTCCTTCAAGAAAAAGCACGT  
 ATTATCAATGACATTTATGCTAATGGAAATGCCCTTCAAAAAAGGTGGCT  
 TCCACCTACCTCGAACTTTCCTTTTTTATTCTGGCTTTTTCACACCTCTTTA  
 CTATTTGGGAAATAATAAGTTAGCAAATGTTGCTGAAATCATTAAATTAAT  
 TATTCGTGATGAATCTGTACATGGTACTTATATCGGTTACAAATTCAGCTT  
 GGTTTTAACGAATTACCAGAAGATGAGCAAGAGAATTTTCGTGATTGGAT  
 GTATGACCTCCTTTATCAGCTGTATGAAAACGAAGAAAAATACACCAAGA  
 CACTTTATGATGGCGTAGGATGGACTGAAGAAGTTATGACCTTTTTACGCT  
 ACAATGCTAATAAAGCTCTTATGAATTTAGGACAAGATCCTTTATTCCAG  
 ATACAGCAAATGATGTCAACCCAATTGTTATGAATGGTATTTCAACAGGAA  
 CATCAAACCATGACTTCTTCTCTCAAGTAGGTAATGGTTACCTACTTGGTA  
 GCGTTGAAGCTATGCATGATGACTATAACTATGGATTATAA

MTTYEAINWNEIEDVIDKSTWEKLTEQFWLDTRIPLSNDLDDWRKLSAQEK  
 DLVGKVFGGLTLLDTMQSETGVEAIRADVTPHEEA VLNNIQFMESVHAKSY  
 SSIFSTLNTKSEIEEIFEWTNNNEFLQEKARIINDIYANGNALQKKVASTYLETF  
 LFYSGFFTPLYLGNKLANVAEIIKLIIRDESVHGTIYIGYKFQLGFNELPEDEQ  
 ENFRDWMYDLLYQLYENEEKYTKTLYDGVGWTEEVMTFLRYNANKALMNL  
 GQDPLFPDTANDVNPIVMNGISTGTSNHDFFSQVGNGYLLGSVEAMHDDDYN  
 YGL\*

Sequence description:

- A] Length: 960 bp - 320 aa (full length gene)
- B] Shine Dalgarno sequence present upstream of  
ATG start codon, but no signal peptide  
present.

ID-98

FIG. 1 CONT'D

21 / 110

Clone RS-77 (partial sequence)

ATGAATTGGTCACGTATCTGGGAACTCGTAAAAATTAATATCCTTTATTCA  
AACCCCTCAGACTCTATCGGCACTAAGAAAAAAGCAAGAAAAGCATCCTAA  
AAAAGAATTTTCAGCTTATAAATCCATGTTTAGAAATCAGTTATTTTCAGAT  
TTTGCTCTTTTCAATAATTTATGTATTTCTCTTTGTATCACTTGATTTTAAAG  
AATATCCGGGCTATTTACGTTCTACATTGGTATCTTTACACTAGTATCCAT  
TATCTACTCTTTTATTGCGATGTACAGTGTTTTCTATGAGAGTGACGATGTT  
AA

MNWSRIWELVKINILYSNPQTL SALRKKQEKHPKKEFSAYKSMFRNQLFQILL  
FSIIYVFLFVSLDFKEYPGYFTFYIGIFTLVSIISFIAMYSVFYESDDV

Sequence description:

- A] Length: 311 bp - 103 aa (Partial sequence)
- B] Shine Dalgarno sequence present upstream of  
ATG start codon, no obvious signal peptide at  
N-terminus.

ID-99

Clone RS-78 (partial sequence)

TAATCTTTTAGTCAACGGAGCAACAGGAAAATTGCAGGCTATGCGACAGA  
TATTCCACCACATAATTTAGCAGAAGTCATTGATGCTGTCGTGTACATGAT  
TGATCACCTAAAGCTAAATTAGATAAATTAATGGAATTTCTACCTGGTCC  
AGATTTTCCAACCTGGCGCTATCATTCAAGGAAAAGATGAAATTCGTAAGG  
CATATGAGACTGGTAAGGGGAGAGTAGCGGTTTCGCTCGCGAACTGCTATT  
GAAACCTTAAAAGGTGGTAAGAAACAAATTATTGTTACTGAAATTCCTTAT  
GAAGTTAAT

SFSQRSNRKIAGYATDIPPHNLAEVIDAVVYMHDPKAKLDKLMEFLPGPDFPT  
GAI IQGKDEIRKAYETGKGRVAVRSRTAIETLKGGKKQIIVTEIPYEVN

Sequence description:

- A] Length: 312 bp - 104 aa (Partial sequence)
- B] No obvious Shine Dalgarno sequence or a

FIG. 1 CONT'D

22 / 110

signal peptide. Both N- and C- termini of ORF  
yet to be elucidated.

ID-100

Clone RS-79

ATGGGACGTAAGTGGGCCAATATTGTTGCCAAAAAGACTGCTAAAGATGG  
TGCTAACTCAAAAAGTATACGCTAAATTCGGTGTTGAAATATATGTTGCTGC  
AAAGCAAGGTGAACCAGACCCCGAGTCAAACCTCAGCTCTAAAATTCGTTT  
TGGACCGTGCTAAGCAAGCACAAAGTTCCAAAGCATGTTATTGATAAAGCG  
ATTGATAAAGCCAAAGGAAACACAGATGAAACTTTTCGTAGAGGGACGCTA  
TGAAGGTTTTTGGTCCAAATGGTTCAATGATTATTGTGGATACTTTGACATC  
AAATGTTAACCGTACGGCAGCAAATGTACGTACTGCTTACGGTAAGAACG  
GTGGCAATATGGGAGCTTCAGGATCGGTATCCTACTTATTTGATAAAAAAG  
GTGTCATCGTTTTTGGTGGTGATGATGCTGACACTGTCTTCGAACAATTACT  
TGAAGCGGATGTAGACGTAGATGATGTTGAAGCAGAAGAGGGAACAATA  
ACAGTTTATACCGCCCCAACAGATCTTCATAAAGGTATCCAAGCACTTCGC  
GATAATGGTGTAGAAGAATTCCAAGTTACTGAACTTGAAATGATTCCTCAA  
TCAGAAGTAGTATTGGAAGGTGATGACCTTGAACTTTTGAAAAGCTT

MGRKWANIVAKKTAKDGANSKVYAKFGVEIYVAAKQGEPPESNSALKFVL  
DRAKQAQVPKHVIDKAIDKAKGNTDETFVEGRYEGFGPNGSMIIVDTLTSNV  
NRTAANVRTAYGKNNGNMGASGSVSYLFDKKGIVVFAGDDADTVFEQLLEA  
DVDVDDVEAEEGTITVYTAPTDLHKGIQALRDNGVEEFQVTELEMIPQSEVVL  
EGDDLETFEKL

Sequence description:

- A] Length: 654 bp - 218 aa (Partial sequence)
- B] Possible Shine Dalgarno sequence upstream  
of ATG start, no obvious signal peptide

ID-101

Clone RS-80

FIG. 1<sub>CONT'D</sub>

23 / 110

TTGGAGAAATATTTGAAGAACCCGATTACATGGATTGGATTAGTTCTTGTG  
GTTACGTGGTTTTTAACTAAAAGTAGTGAATTTTGGATTGTTGGTGTGTGTG  
TCTTGTTGTTAGTATTTGCTAGTCAAAGTGAT

MEKYLKNPITWIGLVLVVTWFLTKSSEFLIFGVCVLLLVFASQSD

Sequence description:

- A] Length: 135 bp - 45 aa (partial sequence)
- B] Shine Dalgarno sequence upstream of TTG  
start codon with possible signal peptide  
evident at N-terminus.

ID-102

Clone RS-81

ATGACACAATCAGATGCATATCTCTCGTTGAACGCGAAGACACGCTTTAGA  
GATCGCACAGGTAATTATCATTTTACTTCGGATAAAGAGGCTGTTGAACAA  
TATATGATAGAACATGTTGAACCTAATACGATGGTGTTCACATCACTAATT  
GAAAAGCTAGATTATTTGGTTTCTAATAACTACTATGAATCGGACCTTCTA  
AAACAATATAACCTTGAGTTTATTTGCCAAATTTTGGAGCATGCATACGCT  
AAGAAATTTGCTTTTCTAAATTTTATGGGGGCTTTAAAATTTTATAATGCTT  
ATGCTCTTAAT

MTQSDAYLSLNAKTRFRDRTGNYHFTSDKEAVEQYMIEHVEPNTMVFTSLIE  
KLDYLVSNYYESDLLKQYNLEFICQIFEHAYAKKFAFLNFMGALKFYNAYA  
LN

Sequence description:

- A] Length: 318 bp - 106 aa (Partial sequence)
- B] Shine Dalgarno sequence present upstream of  
ATG start codon, no obvious signal peptide

FIG. 1<sub>CONT'D</sub>

24 / 110

ID-103

Clone 2-11A

ATGGTATTTATGGCAAATAAGAAAAAACAAGGAAAGAAAACCAGAA  
GACCTACTAAGGCAGAAATAGAGCGTCAAAGAGCTATTCAAAGGATGATT  
ACTGCTCTTGTTTTAACAAATTATTCTCTTCTTTGGTATTATCAGATTAGGTA  
TTTTTGGTATTACAGTCTATAACGTCATCCGTTTTATGGTAGGTAGCTTGGC  
TACTTATTTATTGCGGCAACTTTAATCTACCTTTATTTCTTTAAATGGTTG  
CGAAAGAAAGATAGCTTAGTAGCAGGTTTTTTGATAGCTTCTTTAGGATTA  
TTGATTGAGTGGCATGCTTACCTTTTCTCAATGCCTATTTTGAAAGATAAA  
GAAATTTTGC GTTCAACTGCTCGATTAATTGTGTCTGATTTAATGCAATTTA  
AAATCACTGTTTTTGCCGGTGGAGGTATGTTGGGTGCTTTGATTTACAAGC  
CAATTGCTTTTTCTCTTTTCTAATATTGGTGCCTATATGATTGGTGTTCTCTTC  
ATCATTTTGGGTCTCTTTTTAATGAGTTCTCTGGAAGTTTATGACATCGTCG  
AATTTATTAGAGCTTTTAAAAATAAAGTGGCAGAGAAGCACGAGCAAAAT  
AAAAAGGAGCGTTTTTGCTAAGCGAGAGATGAAAAAAGCAATCGCTGAACA  
AGAGCGCATAGAGCGTCAAAAAGCTGAAGAAGAAGCTTATTTAGCTTCGG  
TTAATGTAGACCCTGAAACGGGTGAGATTCTAGAGGATCAAGCTGAGGAC  
AATTTGGATGATGCGCTACCACCTGAGGTAAAGTGAAACATCAACTCCGGT  
ATTTGAGCCAGAGATCCTTGCTTATGAGACATCGCCTCAAAATGATCCTTT  
ACCAAGTAGAGCCGACAATTTATTTAGAAGACTATGATTCGCCGATTCTTA  
TATGAGAGAAAATGATGAGGAAATGGTTTATGATTTAGATGATGATGTAG  
ATGATAGTGATATAGAAAATGTCGACTTTACACCTAAAACGACACTGGTTT  
ATAAATTACCAACGATAGATTTATTTGCACCAGATAAGCCTAAAAATCAAT  
CCAAAGAAAAGGATTTAGTCCGAAAGAATATCAGAGTTTTAGAAGAAACA  
TTTAGAAGTTTTGGTATCGATGTAAAAGTAGAACGTGCTGAAATTGGACCA  
TCAGTTACTAAATATGAAATTAACCAGCAGTTGGAGTTCGTGTGAATCGT  
ATTTCAAATCTATCTGACGACCTAGCTCTTGCTCTTGACGCAAAAGATGTG  
CGTATAGAAGCACCAATTCCTGGAAAATCATTAAATAGGTATTGAAGTTCCT  
AACTCAGAAATTGCAACGGTTTCTTTCCGCGAACTTTGGGAACAATCTGAT  
GCCAATCCTGAAAACCTTTTAGAAGTACCACTAGGAAAAGCTGTAAACGG  
CAATGCTCGCAGTTTTAACTTAGCTAGAATGCCGCATCTTTTGGTAGCTGG  
TTCAACTGGTTCAGGTAAATCTGTGGCAGTTAATGGAATTATTTCAAGTAT  
TTTGATGAAGGCACGTCCAGATCAAGTTAAGTTTATGATGATTGATCCCAA  
AATGGTTGAATTATCTGTTTATAATGATATTCCACATTTATTAATCCCTGTT  
GTAACCAATCCGCGTAAAGCAAGTAAGGCACTCCAAAAAGTTGTTGATGA  
AATGGAAAATCGATACGAGTTATTTAGCAAAATTGGTGTGCGTAATATAG  
CAGGTTATAATACAAAGGTTGAAGAGTTTAATGCTTCCTCTGAGCAAAAC  
AAATGCCTTTGCCTTTAATCGTTGTCATTGTAGATGAATTGGCTGACTTGAT  
GATGGTTGCTAGTAAAGAAGTTGAAGATGCTATTATTCGTTTGGGGCAAAA  
AGCACGTGCTGCAGGTATCCATATGATTCTTGCAACTCAACGTCCATCCGT

FIG. 1<sub>CONT'D</sub>

25 / 110

AGATGTTATTTCTGGTTTGATTAAAGCAAATGTTCCGTCGCGTATTGCATTT  
 GCTGTTTCAAGTGGTACTGATAGCCGTACGATCCTTGATGAAAATGGTGCT  
 GAAAAGCTCTTGGGACGGGGTGACATGCTCTTTAAGCCTATTGATGAGAAT  
 CATCCAGTACGACTACAAGGTTCTTTATTTTCAGATGATGATGTTGAAAGG  
 ATCGTTGGTTTTATCAAAGACCAAGCCGAGGCTGACTATGATGATGCCTTT  
 GATCCTGGAGAAGTATCTGAAACAGATAACGGCTCTGGTGGTGGCGGCGG  
 AGTACCTGAAAGTGATCCTCTTTTTGAAGAAGCCAAGGGACTCGTTTTAGA  
 GACGCAAAAAGCAAGTGCCTCAATGATTCAACGCCGATTGTCTGTTGGTTT  
 CAATAGAGCAACAAGACTAATGGAAGAATTAGAAGCAGCGGGGGTTATTG  
 GTCCAGCAGAAGGAACCAAGCCACGAAAAGTTTTAATGACTCCAACCTCCG  
 AGTGAATAA

MVFMANKKKTKGKKTRRPTKAEIERQRAIQRMITALVLTIIFFGIIRLGIFGIT  
 VYNVIRFMVGSLAYLFIATLIYLYFFKWLRKKDSL VAGFLIASLGLLIEWHA  
 YLFSPILKDKEILRSTARLIVSDLMQFKITVFAGGGMLGALIYKPIAFLFSNIG  
 AYMIGVLFIILGLFLMSSLEVYDIVEFIRAFKNKVAEKHEQNKKERFAKREMK  
 KAIAEQERIERQKAEIEAYLASVNVDPETGEILEDQAEDNLDDALPPEVSETST  
 PVFEPEILAYETSPQNDPLPVEPTIYLEDYDSPIPNMRENDEEMVYDLDDVDVDD  
 SDIENVDFTPKTTLVYKLPTIDLFAPDKPKNQSKEDLVRKNIRVLEETFRSFGI  
 DVKVERAEIGPSVTKYEIKPAVGVRVNRISNLSDDLALALAAKDVRIEAPIPGK  
 SLIGIEVPNSEIATVSFRELWEQSDANPENLLEVPLGKAVNGNARSFNLARMPH  
 LLVAGSTGSGKSVAVNGHISSILMKARPDQVKFMMIDPKMVELSVYNDIPHLI  
 PVVTNPRKASKALQKVVDENENRYELFSKIGVRNIAGYNTKVEEFNASSEKQ  
 QMPLPLIVVIVDELADLMMVASKEVEDAIIRLGQKARAAGIHMILATQRPVSD  
 VISGLIKANVPSRIAFAVSSGTDSTILDENGAEKLLGRGDMLFKPIDENHPVRL  
 QGSFISDDDVERIVGFIKDQAEADYDDAFDPGEVSETDNGSGGGGGVPESDPL  
 FEEAKGLVLETQKASASMIQRRLSVGFNRATRLMEELEAAGVIGPAEGTKPRK  
 VLMTPTPSE\*

Sequence description:

- A] Length: 2451 bp - 817 aa (Full-length gene)
- B] Shine Dalgarno sequence present upstream of  
 ATG start codon, possesses a potential signal  
 peptide

ID-104

Clone 2-18/22b

FIG. 1 CONT'D

26 / 110

ATGTCACAAGAGCAAGGAAAAATTTATATTGTAGAAGATGATATGACGAT  
 TGTGTCACCTTTTAAAAGATCATTTATCAGCTAGCTATCATGTCTCTAGTGTC  
 AGCAATTTTCGTGATGTGAAACAAGAAATTATCGCATTTCAACCCGATTTG  
 AACTAATGGATATTACGTTACCCTATTTTAATGGTTTTTACTGGACTGCAG  
 AATTGCGTAAGTTTTTAACAATTCCTATTATTTTCATTTTCATCTAGTAATGA  
 TGAAATGGATATGGTTATGGCATTAAATATGGGGGGTGATGACTTTATTTC  
 AAAACCATTCTCTCTAGCTGTATTAGATGCTAAGCTAACTGCTATTTTAAG  
 GAGAAGTCAACAATTTATCCAACAGGAATTAACCTTTTGGGGGATTTACGTT  
 GACAAGAGAAGGGTTATTGTCTAGCCAAGATAAAGAGGTTATTTTATCGC  
 CAACAGAAAATAAAATCCTATCTATCTTGCTCATGCATCCTAAACAAGTAG  
 TCTCAAAAGAGTCTCTATTAGAGAACTTTGGGAAAATGATAGTTTTATTG  
 ATCAAAATACACTTAATGTTAATATGACACGCTTACGTAAAAAAATTGTCC  
 CAATAGGTTTTGATTACATTCATACAGTGAGAGGAGTTGGGTATTTACTAC  
 AATGA

MSQEQGKIYIVEDDMTIVSLLKDHLASASYHVSSVSNFRDVKQEIIAFQPDILM  
 DITLPYFNGFYWTAELRKFLTIPHFISSNDEMMDVMALNMGGDDFISKPFSLA  
 VLDAKLTAILRRSQQFIQQELTFGGFTLTREGLLSSQDKEVILSPTENKILSILLM  
 HPKQVVSKESSLLEKLWENDSFIDQNTLNVNMTRLRKKIVPIGFDYIHTVRGVG  
 YLLQ\*

#### Sequence description:

A] Length: 669 bp - 223 aa (full-length gene sequence)

B] Shine Dalgarno sequence present upstream of a GTG start codon. Was not identified directly by LEEP. This gene was found upstream of gene ID-10 described in WO 00/06736.

ID-105

Clone 2-20

ATGTATCAAACCTCAGACAAATAAGGAAAAATTTGTTTTATTTTTGAAATTA  
 TTTATCCCAGTATTGATTTATCAATTTGCTAATTTTTCAGCTACTTTTATTGA  
 TTCGGTTATGACTGGACAGTATAGTCAGCTACATTTGGCAGGTGTGTCAAC  
 TGCTAGTAATTTATGGACTCCGTTTTTCGCTTTATTAGTAGGTATGATTTCA  
 GCATTAGTACCAGTAGTTGGTCAACATTTGGGTAGAGGAAATAAAGAACA  
 AATTCGCACAGAATTTTCATCAATTTCTATATTTAGGTTTGATACTGTCCTTA  
 ATATTATTTTAAATCATGCAATTTATTGCTCAACCTGTCTTGGGGAGTTTGG

**FIG. 1** CONT'D

27 / 110

GTTTAGAAGATGAAGTTCTAGCAGTTGGTCGTGGTTATTTAAATTATATGT  
 TGATTGGAATCATGCCGCTGGTGTGTTTAGCATTTGCCGTTTCATTCTTTGA  
 TGCATTGGGGTTAACAAGGTTATCTATGTATCTGATGCTTTTAATTCTACCC  
 TTTAATTCATTTTTTAATTATATGCTTATCTACGGTAAATTTGGTATGCCTA  
 GACTAGGAGGTGCGGGGGCAGGTCTTGGAACCTCTTTAACTTATTGGGCTA  
 TTTTATTGGTATTATTATTGTGATGTCACTTCATCCTCAAATTAACATA  
 TCATATATGGACTCTGGAAAGAATAAAAGCTCCTTTGATTATTGAAGATAT  
 TCGATTGGGATTACCGATTGGTTTACAAATTTTGCAGAAGTTGCAATTTT  
 GCAGTAGTAGGCTTATTCATGGCAAAATTTCTTCAATCATTATTGCAGCA  
 CATCAGGCTGCTATGAATTTTTCATCATTAAATGTATGCATTTCCTTTAAGTA  
 TTTCCACTGCTCTAGCTATTACAATATCGTTTGAAGTAGGGGCAGAGCGCT  
 TTCAGGACGCAACCACTTATAGTAGGATAGGACGCTTAACAGCGGTAGGG  
 ATTACATCAGGAACCTTACTATTTTTATTTCTATTTTCGTGAGAATGTAGCAG  
 CAATGTATAATAGTGCCCTCACTTTGTGCTATTACAGCTCAATTCCTAAC  
 TTATAGTCTCTTTTTCCAGTTTGCAGATGCTTATGCAGCTCCTGTACAGGGG  
 ATTTTACGAGGCTATAAGGATACAACAAAACCATTTATGATCGGTGCGGG  
 CTCTTATTGGTTATGTGCTTTGCCATTAGCGGTTATCTTAGAAAAAATAG  
 CCAGTTAGGTCCGTTTGCCTATTGGATTGGTTTAATCACAGGTATTTTTGTT  
 TGTGGTCTATTTCTAAACCAACGTCTGCAAAAGATTAAGAAGTTGTATTAT  
 TAA

MYQTQTNKEKFVFLKLFIPVLIYQFANFSATFIDSVMTGQYSQLHLAGVSTAS  
 NLWTPFFALLVGMISALVPVVGQHLGRGNKEQIRTEFHQFLYLGLLSLILFLI  
 MQFIAQPVLGSLGLEDEVLA VGRGYLNYMLIGIMPLVLF SICRSFFDALGLTRL  
 SMYLMILLIPNSFFNYMLIYGKFGMPRLGGAGAGLGTSLTYWAIFIGIIVMS  
 LHPQIKTYHIWTLERIKAPLIIEDIRLGLPIGLQIFAEVAIFAVVGLFMKFSSIIA  
 AHQAAMNFFSLMYAFPLSISTALAITISFEVGAERFQDATTYSRIGRLTAVGITS  
 GTLLFLFLFRENVAAMYN SAPHVAITAQFLTYSLFFQFADAYAAPVQGILRG  
 YKDTTKPFMIGAGSYWLCALPLAVILEKNSQLGPFAYWIGLITGIFVCGLFLNQ  
 RLQKIKKLYY\*

## Sequence description:

- A] Length: 1341 bp - 447 aa (full length gene)
- B] Shine-Dalgarno sequence present upstream of  
ATG start codon, There is a potential signal  
peptide sequence

ID-106

FIG. 1<sub>CONT'D</sub>

28 / 110

Clone 2-4A

TTGCTAGTTTCTTCTCTAGTTTCTTGTTCAATTTTTCTTGTCATTTTCGTCGTT  
GTCTTCATCAACACGAAATAAGTCTATAAACTTATCAAATAATTTCATAGA  
CTTATTATATCAATTTTCAATAAAATGCTATAATAAAACCATGTCATTTTCA  
TTAAAAATTAGAAATCCATACGGTGAACATACCGTTAAAGAAGTCTCTGA  
AGATTATTTTTTGATTCCACGTAAGATTAGACATTTTTTTCGTGTTAAAAAA  
CATGTACTTATAAACAATGAATTCATTAATTGGCAAAGTTCGTCCAAGAA  
AACGATACTATTACCTTAATCTTTGATGATGAGGATTACCCTACTAAAAAA  
ATTCCTCTGGGCAGAGCAGAGCTTATTGATTGTCTTTATGAGGATGAACAT  
CTTATTATCGTTAATAAACCTGAAGGTATGAAAAGTACCGGTAACCAACCA  
AATGAAATAGCACTGTAAATCATGTATCTGCCTATTCTGGACAAACATGC  
TATGTTGTTTCATCGCCTAGATATGGAGACCAAGTGGAGCTGTTTTATTTGCT  
AAAAATCCATTTATACTTCCCCTTATCAATCAACGCTTAGAACGAAAAGAA  
ATTTGGCGTGAATATTGGGCTTTAGTTGAAGGAAAATTTTACCTAAGCAT  
CAAGTTTTGAGAGACAAAATTGGACGGAACCGTCATGACAGACGTAAACG  
AATCATTGATTCTAAAAACGGTCAACATGCTATGACAATCATTGACGTTTT  
GAAGTATATCCAAAATAGTAGTCTCATAAAATGCCGACTGGAAACCGGAA  
GAACCCATCAAATTCGCATTCATTATCTCATCACGGACATCCTTTAATAG  
GAGATCCCCTCTACAACCCTTCTTCTAATAATGAAAGGTTAATGCTACACG  
CTCACCGATTGACTCTATCCCATCCATTAAGTTCGCGAACTATTAGCGTAG  
AGGCCCTTCATCTACTTTTCGAGAAGGTTTTAAACAATTATAAAAAAGGAG  
TTGGATAA

MLVSSLVSCSFFLVISSLSSSTRNKSINLSNMFIDLLYQFSIKCYNKTMSFSLKIR  
NPYGEHTVKELLEDYFLIPRKIRHFLRVKKHVLINNEFINWQTVVQENDTITLIF  
DDEDYPTKKIPLGRAELIDCLYEDEHLIIVNKPEGMKTHGNQPNIEALLNHVSA  
YSGQTCYVVHRLDMETSGAVLFAKNPFIPLINQRLERKEIWREYWALVEGKF  
SPKHQVLRDKIGRNRHRRRKRIIDSKNGQHAMTIIDVLKYIQNSSLIKCRLETG  
RTHQIRIHLSHHGHPLIGDPLYNPSSNNERLMLHAHRLTLSHPLTCETISVEAPS  
STFEKVLNNYKKGVG\*

Sequence description:

- A] Length: 1029 bp - 343 aa (Full length gene sequence)
- B] No obvious Shine-Dalgarno sequence upstream of the putative TTG start codon. Possesses a potential leader peptide sequence.

FIG. 1<sub>CONT'D</sub>

29 / 110

ID-107

Clone 2-54

GAACTAAATGCAACTCAACCTAATAATAGAACTACCTATATTATACCCGAA  
AGCAGTCATTCCATTGCAGAACACAGAGATTCCTGATAGAATCAAAGGG  
TTCTTCGGTTGCATTACTTAATAGCGATGAATTTAGAAAGACAGCGGGAGA  
GGATAGAGGTTTTGAAAGGGATAAGTTGAGGTCTTTGGATATCATTCTAA  
GGGAGATTTATCGACAAGTAATGTCATAGGTAATACGGACATTGCTAGTC  
AGATATCGTTGGGCTTTAAAAAGAATGCGATGCAGGAACACCATCTTACT  
AAAACATTCTCTCAAAGGATGGAAAGTTATCGTCTGTTATAGAGGGGAT  
GCTTGCTATTGGCAAAGAGAAAGTAGAGAAAGAAATAAAATATAGTGGTA  
ATTTATGGCAAAAATTAAAAGCTAAGGCACACTGCCTTGTTTGCTGTGTTG  
ATAATTTGAATTTTGAAGATATAAAATCTTATTTTCAATATTATTGTCATCT  
AAACCATCAGCTCAAATTACCTAAAGGTGCTATACTTTCTGCTAAAACAGA  
AGTATATAGGGGAGGAGATTTTGGGAGAAAAAATAAAGATAATGTGTTTG  
GTTACCGTATCCCCCTCATTATTGAAAACCCAAAAAGGAACTTTACTTGCGG  
GAGCTGATGAAAGAATTGAGCAAGCTTGTGATTGGGGAAACATAGGAATG  
GTTATTCGCCGTAGTGAGGATGATGGTGTCACTTGGGGAAAAAGAGAAAC  
TATTGTCAATCTCCGTAATAACCCTAGAGTTCCGCTAGTTACTAGTGGTGA  
CTATAGTGGCTCACCTATTAATATGGATATGGCATTAGTTCAAGATACTAG  
CTCCAAGACGAAACGTATTTTTTCAATATATGATATGTTTCCAGAAGGAAG  
AGGCGTTATTAGTATTGCTAACACACCTGAAAAAGAATATACCCAAATCG  
GAGGACAGTCTTATCTTAATTTATATAATAATGGAAAGAAATCGAAGGTTT  
TACTATCCGTGACAAAGGTATTGTATATAATTTTAAAGGGAAAAAGACTG  
ATTATCATGTTATAACAGAACTACTAAAAGTGACCATTCAAATCTAGGGG  
ATATTTATAAGGGAAAAACAGCTACTTGAAATATATATTTTACAAAACATA  
AAACGTCACCATTTTCGTTTAGCAAAATCAAGCTATGTGTGGATGTCATATA  
GCGATGATGATGGTAGGACATGGTCATCACCTAGAGATATAACAGCAAGT  
CTTCGTCAGAAAGGCATGAAATTTTTGGGAATAGGACCTGGAAAAGGTAT  
AGTTTTAAATGGGGGCCACACGCTGGTCGTATTATTATTCCTGCCTATTCT  
ACGAATTGGAAATCTCATCTAAGAGGTTCAACAATCTTCACGCCTAATTTAT  
TCAGACGACCATGGAAAAACGTGGCATACTGGAAAAGCAGTTAATGATAA  
CCGTATACTTTCTAATGGTGAAAAAATCACTCCTTAACAATGGATAATAA  
AAAAGAACAAAATACAGAATCCGTACCCGTTCAATTGAAAAATGGGGACA  
TTAAGTTATTTATGAGGAATCTAACTGGTAACCTAGAAGTAGCCACAAGTA  
AAGACGGCGGGGAGACTTGGCAAAACCATGTAAACGATATAAGGAAATT  
CATGATGCTTACGTCCAACCTATCAGCTATTCGCTTTGAGCATGACAAAAA  
GAGTATATTTTATTAGTGAATGCTAATGGGCCAGGGAAGAAGTGCCAAGA  
TGGATATGCACGTCTAGCGCAAGTTAATCGAAATGGTAGTTTTAAGTGGTT  
ATATCACCATCACATTCAAGATGGTTCGTTTGCTTACAACCTCTGTTCAACA  
ACTTAATAATGATCAATTTGGTGTCTTTTATGAACATAGAGAAAAACATCA

FIG. 1<sub>CONT'D</sub>

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AAATAGTTTTACTTTAAATTACAAAGTTTTTAATTGGAGTTTTCTTAGTCAA  
 AATACAGAGAAGCAAGGCACTTTATGGGAGAAAATGGCAGCAAATTGGCA  
 TGTTTTGTTTAAATTTTATTTATGA

ELNATQPNRRTTYIIPESHSIAEQQRFLIESKGSSVALLNSDEFKRTAGEDRGF  
 ERDKLRSLDIIPKGDLSNVIGNTDIASQISLGFKKNAMQEHLTKTFSQKDG  
 KLSSVIEGMLAIGKEKVEKEIKYSGNLWQKLKAKAHCLVCCVDNLNFEDIKS  
 YFQYYCHLNHQLKLPKGAILSaktevyrGGDFGRKNKDNVFGYRIPSLKTO  
 KGTLLAGADERIEQACDWGNIGMVIRSEDDGVTWGKRETIVNLRNNPRVPL  
 VTSGDYSGSPINMDMALVQDTSSKTKRIFSIYDMFPEGRGVISIANPEKEYTQI  
 GGQSYLNLNNGKKSKVFTIRDKGIVYNFKGKKTDYHVITETTKSDHSNLGDI  
 YKGKQLLGNIYFTKHKTSFRLAKSSYVWMSYSDDDGRTWSSPRDITASLRQ  
 KGMKFLGIGPGKGIVLKWGPHAGRIIPAYSTNWKSHLRGSQSSRLIYSDDHG  
 KTWHTGKAVNDNRILSNGEKIHSMTDNKKEQNTESVPVQLKNGDIKLFMRN  
 LTGNLEVATSKDGGETWQNHVKRYKEIHDA YVQLSAIRFEHDKKEYILLVNA  
 NGPGKKCQDGYARLAQVNRNGSFKWLYHHHIQDGSFA YNSVQQLNNDQFG  
 VLYEHREKHQNSFTLNYKVFNWSFLSQNTTEKQGTLEWMAANWHVLFKFYL  
 \*

Sequence description:

- A] Length: 2052 bp - 684 aa (partial gene sequence)
- B] N-terminus has yet to be determined

ID-108

Clone 2-61

ATGCCTAAATTAATCGTATCTTTCCTCTGCATTTTATTATCCCTGACTTGTG  
 TAAACTCTGTGCAAGCTGAAGAACATAAAGATATTATGCAAATTACCCGA  
 GAAGCCGGATATGATGTTAAAGATATTAATAAACCTAAAGCGTCTATCGTT  
 ATTGACAATAAAGGTCATATTTTGTGGGAAGATAACGCCGATTTAGAACGT  
 GATCCCGCTAGCATGTCTAAAATGTTTACTTTATATTTACTATTTGAAGACT  
 TAGCTAAAGGAAAAACAAACCTCAACACCACAGTGACTGCAACAGAAACA  
 GACCAAGCCATAAGTAAGATTTATGAAATTAGTAATAACAATATTCATGCT  
 GGGGTTGCTTATCCTATTCGTGAAGTACTATGACGGCTGTCCCGTCA  
 TCTAATGTAGCAACTATTATGATTGCTAACCCTTATCACAAAACAATCCT  
 GACGCCTTTATTAAACGAATCAATGAAACCGCCAAGAACTCGGTATGAC  
 AAAAATCACTTTTATAACCCCAAGTGGGGCGGTAGCGAGTGCTTTTAATGG  
 ACTTTACTCCCCAAAAGAATACGATAACAATGCTACTAACGTTACGACTGC

FIG. 1<sub>CONT'D</sub>

31 / 110

ACGTGATCTATCAATTTTAACCTATCATTTCCTTAAAAAATACCCTGATATA  
CTGAACTATACAAAATATCCTGAAGTCAAGGCCATGGTCGGAACCTCTTAT  
GAAGAAACATTTACAACCTATAACTACTCTACCCCCGGCGCTAAATTTGGA  
TTAGAAGGAGTAGATGGCTTAAAAACTGGTTCTAGCCCTAGCGCTGCTTTT  
AATGCCTTAGTTACAGCTAAACGCCAGAATACTCGCTTGATAACTGTGGTT  
TTAGGAGTTGGCGATTGGTCAGACCAAGACGGAGAGTACTATCGTCATCC  
GTTTGTCAACGCTCTTGTAGAAAAAGGTTTTAAAGACGCTAAAAATATTTT  
TTCTAAAACCTCCTGTATTAAGCCGTTAAACCTAAAAAAGAAGTTACTAA  
AACCAAACTAAATCTATTCAAGAACAGCCTCAAACAAAAGAACAGTGGT  
GGACAAAACAGATCAATTTATCCAATCACATTTTGTATCTATTTTAATTG  
TTCTGGGCACCATCGCTAGCCTTTGTCTTTTAGCTGGGATAGTATTACTTAT  
AAAGCGCTCTAGATAA

MPKLIVSFLCILLSLTCVNSVQAEHDKDIMQITREAGYDVKDINKPKASIVIDN  
KGHILWEDNADLERDPASMSKMFTLYLLFEDLAKGKTNLNTTVTATETDQAI  
SKIYEISNNNIHAGVAYPIRELITMTAVPSSNVATIMIANHLSQNNPDFAIKRINE  
TAKKLGMTKTHFYNPSGAVASAFNGLYSPKEYDNNATNVTTARDLSILTYHF  
LKKYPDILNYTKYPEVKAMVGTPYEETFTTYNYSTPGAKFGLEGVDGLKTGS  
SPSAAFNALVTAKRQNTLITVVLGVGDWSDQDGEYYRHPFVNALVEKGFK  
DAKNISSKTPVLKAVKPKKEVTKTCTKTSIQEQPQTKEQWWTKTDQFIQSHFVS  
ILIVLGTIASLCLLAGIVLLIKRSR\*

Sequence description:

- A] Length: 1188 bp - 396 aa (full length gene)
- B] Shine Dalgarno sequence present upstream of  
ATG start codon, possesses a potential signal  
peptide

ID-109

Clone 45

ATGACTGAAAAATATTATAATTGGGCAACGCTTGGAACCGGCGTTATTGCC  
AACGAATTAGCCCAAGCACTGGAAGCACGTGGACAAAAATTATATTCTGT  
AGCTAATAGAACTTACGACAAAGGACTTGAATTTGCTAACAAATATGGTA  
TCCAAAAAGTTTATGATCACATAGATCAAGTATTTGAAGACCCTGAAGTGG  
ATATCATTATATCTCTACTCCCCACAATACTCACATCTCATTTTACGAAA

FIG. 1<sub>CONT'D</sub>

32 / 110

GGCTTTAGCAAATGGTAAGCACGTTCTTTGCGAAAAATCTATTACTTTAAA  
 TAGTACTGAGCTTAAAGAAGCCATAGATTTAGCCGAAACTAACCATGTTGT  
 CTTAGCTGAAGCCATGACTATTTTTTCATATGCCAATTTACCGCCAATTAAA  
 AACATTAGTTGATAGTGGAAAATTAGGACCGTTAAAAATGATTCAAATGA  
 ATTTTCGGAAGTTATAAAGAATATGATATGACTAACCGTTTTTTTCAGTCGTG  
 ACCTAGCAGGCGGTGCTTTGCTGGACATTGGTGTTTATGCACTTTCTTGAT  
 TCGCTGGTTTATGTCAGAAGCACCTCACAACATTACCTCTCAAGTTACATT  
 TGCACCAACAGGGGTTGATGAACAAGTTGGTATCCTACTAACCAACCCAG  
 CAAATGAGATGGCGACTGTCAGCCTTAGTTTACATGCAAAACAACCTAAA  
 CGAGCAACTATCGCTTACGATAAAGGCTACATTGAACTTTTTGAATATCCG  
 CGAGGACAAAAGGCAGTTATTACTTATACTGAGGATGGGCATCAAGATAT  
 TATCGAAGCTGGCAAACTGAAAATGCTCTCCAATATGAGGTAGCTGATA  
 TGGAAGAAGCCATTTTCAGGAAAACTAACCACATGTACTTAACTATAACC  
 AAAGATGTTATGGATATCATGACACAGCTACGTCAAGAATGGGGATTAC  
 CTACCCAGAAGAAGAAAAATGA

MTEKYYNWATLGTGVIANELAQALEARQKLYSVANRTYDKGLEFANKYGI  
 QKVYDHDIDQVFEDPEVDIIYISTPHNTHISFLRKALANGKHVLCEKSITLNSTEL  
 KEAIDLAETNHVVLAEAMTIFHMPYRQLKTLVDSGKLGPLKMIQMNFGSYK  
 EYDMTNRFFSRDLAGGALLDIGVYALSCIRWFMSEAPHNITSQVTFAPTGVDE  
 QVGILLTNPANEMATVSLSLHAKQPKRATIAYDKGYIELFEYPRGQKAVITYT  
 EDGHQDIIIEAGKTENALQYEVADMEEAISGKTNHMYLNYTKDVMDIMTQLR  
 QEWGFTYPEEEK\*

## Sequence description:

- A] Length: 984 bp - 328 aa (full length gene)
- B] Shine Dalgarno sequence present upstream of  
 ATG start codon, possesses a potential signal  
 peptide

ID-110

Clone 2-2

GTGTATTCTCCTGTAAATCTTCTAAAGGAAAAGTGATATTGTAAAAAGT  
 GATTTTCTAAAGAGCTTCATAGAAAGGAGAGGAAATATTTGTTTT

MYSPVKSSKGKVILLKSDFLKSFIERRGNICF

**FIG. 1** CONT'D

33 / 110

## Sequence description:

- A] Length: 96 bp - 32 aa (partial sequence)
- B] GTG start codon - no obvious Shine-Dalgarno sequence
- Possesses a potential signal peptide

ID-111

Clone 2-3

AAATACTGTATCATTGCAACCTCAAATGCAGGTTTTGGAAACGAAGCATTT  
ACAGGTGACAGCGATAAAGACTTGAAAATTATGGAACGAATTTCTCCATA  
TTTCCGTCCAGAATTTCTAAATCGTTTCAATGGTGTTATTGAATTCTCTCAC  
CTAAGCAAAGATGACTTAAGCGAAATTGTAGATTTGATGCTTGATGAAGTT  
AACCAAACAATTGGCAAAAAAGGAATTGACCTTGTGGTAGATGAAAATGT  
TAAATCACACTTAATTGAACTGGGTTATGACGAAGCAATGGGAGTACGTC  
CATTGCGCCGTGTCATCGAGCAAGAAATTCGAGATCGCATCACAGACTACT  
ATCTCGATCATAACAGACGTTAAACACCTAAAAGCTAATTTGCAAGATGGCC  
AAATCGTCATTTCTGAAAGATAA

KYCHATSNAFGNEAFTGDSKDLKIMERISPYFRPEFLNRFNGVIEFSHLKSD  
DLSEIVDLMLDEVNQTIGKKGIDLVVDENVKSHLIELGYDEAMGVRPLRRVIE  
QEIRDRITDYYLDHTDVKHLKANLQDGQIVISER\*

## Sequence description:

- A] Length: 429 bp - 143 aa (partial sequence)
- B] N-terminus yet to be elucidated. This gene was not in frame with nuc

ID-112

Clone 2-5

FIG. 1 CONT'D

34 / 110

ATGTCAATGAATTTTTCATTTTACCACAATATTGGTCCTATTTTAATTATG  
 GTGTGATGGTAACCATTATGATTTCAACATGTGTTGTTTTTTTGGAACTAT  
 TATAGGCGTGTTAATTGCTTTAGTAAAGCGTACTAATTTACATTTTCTCACA  
 ATATTAGCTAATTTCTATGTATGGGTATTTTCGTGGGACACCGATGGTAGTT  
 CAAATTATGATTGCTTTTCGCATGGATGCATTTTAACAATTTACCAACAATT  
 AGCTTTGGTGTTTTAGATTTAGATTTTACACGACTTTTACCTGGTATCATT  
 TCATTTCTTAAATAGTGGTGCCTATATTTTCGGAAATTGTACGTGCAGGGA  
 TTGAGGCTGTACCATCTGGACAAATAGAAGCAGCTTACTCGTTGGGGATT  
 GACCTAAAAATACACTTCGCTATGTTATCTTACCCCAAGCTTTTAAAAATA  
 TTTTACCTGCTCTAGGGAATGAATTTATTACAATTATTAAAGATAGTGCTCT  
 CCTTCAAACCTATTGGTGTCTATGGAATTATGGAACGGAGCACAATCAGTTGT  
 AACGGCTACTTACTCACCAGTTGCACCGTTATTATTTGCAGCATTTTACTAT  
 TTAATGTTGACAACGATTCTCTCAGCTTTGTTAAAACAAATGGAGAAATAT  
 CTTGGGAAAGGGGTAAAAATAGATGGTTGA

MSMNFSFLPQYWSYFNYGVMVTIMISTCVVFFGTIIGVLIALVKRTNLHFLTIL  
 ANFYVWVFRGTPMVVQIMIAFAWMHFNNLPTISFGVLDLDFTRLLPGIIISLNS  
 GAYISEIVRAGIEAVPSGQIEAAYSLGIRPKNTLRVILPQAFKNILPALGNEFITI  
 IKDSALLQTIGVMELWNGAQSVVTATYSPVAPLLFAAFYYLMLTTILSALLKQ  
 MEKYLKGKGVKIDG\*

#### Sequence description:

- A] Length: 699 bp - 233 aa (full length gene)
- B] Shine-Dalgarno sequence preceded the 'ATG' start codon. Possesses a potential leader peptide sequence.

ID-113

Clone 2-7

ATGAAAGACCTATTACGAAATAGTCTAGAGCAAAGTGGAATTTAAGTTT  
 TCAAGATATGATTTTACATATTCTTGTAGCAGCTTTATTGAGTGTTAGTTATT  
 TATGTTTCCTATGCTTATACGCATAGTGGAAGTGCCTATAGTAAAAAGTTT  
 AATGTTTCATTAATGACATTGACGGTCTTGACTGCAACAGTAATGACCGTT  
 ATTGGTAATAATGTAGCCTTGTCATTGGGTATGGTCGGTGCCTTGTCAGTT  
 GTTCGTTTTAGGACAGCCATAAAAGATTCAAGAGATACAGTTTATATTTT  
 TGGACCATAGTTGTTGGTATCTGTTGTGGTGTCTGGTACTATGTGGTAGCT

# FIG. 1<sub>CONT'D</sub>

35 / 110

GCATTAGGAAGTAGCGTTATCTTTATCTTATTATGGGTTATGGGACGTGTT  
AAAAACGAGAATCGTATGTTATTGATTGTGAAGTGCGATAGAACACTAGA  
AGTTGATTTAGAAAGGAATTTTCTTCCAATATTTTGACGGAAAAGCTGTTCA  
GCGTGTTAAAAATTCAACAATACTATTGAAATGATTTTCGAAATCTC  
TAGAAAAGATTACGATAAGCAACTCCATGTAGATAATCAGTTAACTGAAA  
AAGTGTACCAATTGGGAAATATTGATTATTTCAACATTGTTAGCCAAAGCG  
ACGAAATCAATGGGTAG

MKDLLRNSLEQSGNLSFQDMILHILVAALLSVVIYVSYAYTHSGTAYSKKFNV  
SLMTLTVLTATVMTVIGNNVALSLGMVGALSVVRFRRTAIKDSRDTVYIFWTIV  
VGICCGVGDYVVAALGSSVIFILLWVMGRVKNENRMLLIVKCDRTLEV DLEGI  
FFQYFDGKAVQRVKNSTNTIEMIFEISRKYDYDKQLHVDNQLTEKVYQLGNID  
YFNIVSQSDEING\*

Sequence description:

- A) Length: 678 bp - 226 aa (full-length gene)
- B) ATG start codon is preceded by a Shine-Dalgarno sequence-Possesses a potential leader peptide sequence

ID-114

Clone 2-8

AAAAATTCATTTTAGATTTCATTTTACGACTATATACTCAGAAGTACCAAAC  
CTAATCCAAGGTTTGAAAAAAGAAAGAAGGAAGTCAGTATGACAACTAT  
AAAAACAACTTTAAAGATGAGGCTATACGTGTTGAAGAGACAACAAAAGA  
ATCATTTTACGATGTTGATATTGCCTTGTTTTTCAGCTGGTGGATCTATTTCA  
GCAAAGTTCGCTCCTTATGCAGTAAAGTCTGGAGCAGTTGTAGTAGATAAC  
ACGTCATATTTTCGTCAGAATCCTGATGTTCCACTAGTTGTTCTCCTGAAGTAA  
ATGCTCATGCCATGATTGGTCATAATGGTATCATAGCTTGTCCTCAATTGTTT  
TACTATTCAAATGATGATTGCTTTAGAGCCCATTCGTCAAAAATGGGGGAT  
AGAGCGTGTTATAGTTTCCACCTATCAAGCTGTTTCGGGTTTCAGGTGCACG  
TGCTGTTGAAGAACTAAGGAACAGTTGAGACAAGTTTT

KFILD SFYDYILRSTKPNPRFEKRKKEVSMTNYKNNFKDEAIRVEETTKESFYD  
VDIALFSAGGSISAKFAPYAVKSGAVVVDNTSYFRQNPDPVPLVVPEVNAHAMI  
GHNGIIACPNCSTIQMMIALEPIRQKWGIERVIVSTYQAVSGSGARAVEETKEQ  
LRQV

FIG. 1<sub>CONT'D</sub>

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## Sequence description:

- A] Length: 499 bp - 165 aa (partial sequence)
- B] N-terminus has yet to be determined

ID-115

Clone 2-9

ATGACAAATGAATTGATAATGCAAGCTTTTGAGTGGTATTTACCTAGTGAT  
GGGAATCACTGGAAGAAATTAGAGGAGTCTATATCAGACCTTAAAAAACT  
TGGAATTAGTAAAATCTGGTTACCACCAGCATTTAAGGGAAGTAGCAGTG  
ATGATGTAGGATATGGTGTGTTATGATCTCTTTGATTTAGGAGAATTTGACC  
AGAATGGAACAATTAGAACAAAATATGGTAGGAAAGAAGAGTATCTAAA  
GCTTATTAAGTCGTAAAGGCCAAATGGCATTAAACCGTTTGCAGATATCGT  
TCTTAACCATAAAGCCAATGGTGATCATAAAGAAAAATTTCAAGTCATCA  
AAGTCAATCCTGAAAATCGTCAAGAAGCATTAAAGTGAACCCTATGAGATT  
GAAGGATGGACGGGATTTGATTTCCCAGGTAGACAGGGTGAGTACAATGA  
TTTT

MTNELIMQAFEWYLPDGNHWKKLEESISDLKKLGISKIWLPPAFKGTSSDDV  
GYGVYDLFDLGEFDQNGTIRTKYGRKEEYLKLIKSLKANGIKPFADIVLNHKA  
NGDHKEKFQVIKVNPNENRQEALSEPYEIEGWTGFDFPGRQGEYNDF

## Sequence description:

- A] Length: 456 bp - 152 aa (partial sequence)
- B] ATG start codon is preceded by a Shine-Dalgarno sequence, no leader peptide sequence.

ID-116

Clone 2-10

FIG. 1<sub>CONT'D</sub>

37 / 110

ATGGAGGTTCTTATGAAGAAAGTGTTAGTAAGTAGTCTTTTGGTTTTAGGG  
ATTACGATAACGTTACAACCAGTAGTTGAGGCTAAGGGGCCAAAAGTAGC  
TTATACACAAGAGGGAATGACTGCTCTTTCGGACACAAATAAAGATAAAG  
TCACTACTATTTCTATTGACGAGATTCAAAAAAGCTTAGAAGGTAAGAAGC  
CGATTACTGTTAGTTTTGATATTGATGATACACTGCTTTTCAGTAGTCAATA  
TTTTCAATATGGTAAAGAATATGTAACCTCCTGGATCGTTTGATTTTCTTCAT  
AAACAAAAATTCTGGGATCTTGTTGCAAAACGAGGAGATCAAGATTCCAT  
TCCCAAAGAATATGCTAAAAAATTAATTGCTATGCATCAAAAACGAGGAG  
ATAAAATTGTTTTTATAACAGGTAGGACAAGAGGGTCAATGTATAAGGAG  
GGCGAGGTTGATAAAACAGCTAAAGCCTTAGCTAAAGATTTTAAATTTGTA  
CCATCTGAT

MEVLMKKVLVSSLLVLGITITLQPVVEAKGPKVAYTQEGMTALSDTNKDKVT  
TISIDEIQKSLEGKKPITVSFDIDDTLLFSSQYFQYGKEYVTPGSFDLHKQKFW  
DLVAKRGDQDSIPKEYAKKLIAMHQKRGDKIVFITGRTRGSMYKEGEVDKTA  
KALAKDFKFVPSD

Sequence description:

- A] Length: 516 bp - 172 aa (partial sequence)
- B] ATG start codon is preceded by a Shine-Dalgarno sequence, Possesses a leader peptide sequence.

ID-117

Clone 2-17

ATGCTTAAAAGATTATTTACTGAAGATGGGGAATTGACAAAGATTAGTCGT  
CGTTTCGTTTGGATGTTAGTGGTTATCTATTGTCTTATTATTGTCAGGATGT  
GTTTTGGGCCTCAAATTATGATTGAGGGGGTATCAACTCCGAATGTTTCAGC  
GCTTCGGAAGAATTGTAGCTCTTTTAGTACCATTTAATTCTTTTCGTAGTTT  
AGATCAGCTAACTAGCTTTAAAGAGATTCTTTGGGTTATTGGTCAAAATGT  
AGTGAATATTTTACTGCTGTTTCCTCTCATTATAGGGTTACTATCCCTAAAG  
CCAAGTTTACGGAAATATAAAAGCGTTATATTACTTGCTTTCTTGATGTCTC  
TTTTCATAGAGTGTACTCAAGTTGTTTTAGATATTTTAATAGATGCTAATCG  
GGTTTTTGAAATCGACGATCTATGGACAAATACCTTAGGCGGTCCTTTTCGC  
CCTATGGAGTTATCGAAACATAAAAGGTTGGCTTCTAACTATTAGAAAATG  
A

FIG. 1<sub>CONT'D</sub>

38 / 110

MLKRLFTEDGELTKISRRFVWMLVVIYCLIIVRMCFGPQIMIEGVSTPNVQRFG  
RIVALLVPFNSFRSLDQLTSFKEILWVIGQNVVNILLFPLIIGLLSLKPSLRKYK  
SVILLAFLMSLFIECTQVVLDILIDANRVFEIDDLWTNTLGGPALWSYRNIKG  
WLLTIRK\*

Sequence description:

- A] Length: 516 bp - 172 aa (full-length gene)
- B] ATG start codon is preceded by an Shine-Dalgarno sequence. Possesses a potential leader peptide sequence. C-terminus need further confirmation.

ID-118

Clone 3-3

ATGAAAAAGCTTACTTTTATTTGGGATTTAGATGGGACATTAATAGATTTCG  
TATGTACCAATTATGGAAGCTCTTGAAGAAACCTATCGTCATTTTGGCTTA  
ATATTTGATAAAGAATTAATCCATGAATATATTTTACAGGAATCAGTGGGG  
CAATTATTGGTAAACCTTTCAGAGGAAGAGCAAATACCTCATGAAAACT  
GAAAGCATATTTTACAAAAGAACAAGAAAGTCGAGATTCTAAAATACATT  
TAATGCCATATGCAAAAGAGATTTTAGAATGGACCAAAGAACAAGATATT  
CCCAATTTTATGTATACACATAAAGGAGCAAGTACGCATTTCAGTGTTGGAA  
ACCTTGCAGATCTCTCATTATTTTGATGAAATTTTAACTGGTGTTCGGGAT  
TCGAGCGAAAACCATCCACAAGGGATTAATTATTTAGTTAAACGATATT  
CTTTAGATAAATCAATGACTTATTACATAGGAGATCGTCCACTAGATTTGG  
AGGTTGCTCAAAATGCTGGTATAAAATCCATAAACTTAAGGTTAGAGAATT  
CCAAAGAAAACCTATAATATTTCAAGTCTCAAAGATATAATATCACTTGATT  
TCACTCGTTTGGATTAA

MKKLTFIWDLTGTLIDSYVPIMEALEETYRHFGLIFDKELIHEYILQESVGQLL  
VNLSEEEQIPHEKLKAYFTKEQESRDSKIHLMPYAKEILEWTKEQDIPNFMYTH  
KGASTHSVLETLQISHYFDEILTGVSGFERKPHPQGINYLVKRYSLDKSMTYYI  
GDRPLDLEVAQNAGIKSINLRENSKENYNISSLKDIISLDFTRLD\*

Sequence description:

- A] Length: 627 bp - 209 aa (Possible Full-length gene)

FIG. 1<sub>CONT'D</sub>

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B] ATG start codon is preceded by an possible  
Shine-Dalgarno sequence. No obvious leader  
peptide sequence.

ID-119

Clone 3-7

ATGGAAAAAGAAAAAAATTAGGTCTTTTACCACTAACAATGCTTGTCATT  
GGCTCTCTTATCGGTGGCGGAATCTTTGATTTAATGCAAAATATGAGTTCC  
AGAGCCGGTTTGGTACCAATGCTTATTGCTTGGGTAATTACTGCTATCGGG  
ATGGGAACCTTCGTTTTAAGTTTTCAAAATTTATCTGAAAAAGGCCGGAC  
CTAACAGCTGGAATCTTTAGTTACGCTAAAGAGGGGTTTGGAACTTTATG  
GGATTAACTCTGCATGGGGTTATTGGTTATCAGCTTGGCTTGGAAATGTT  
GCCTACGCTGCACTCTTATTCAGTTCACCTCGGTTATTTCTTTAAATTCTTTG  
GTAATGGAAATAATATCATCTCAATTATTGGAGCAAGTATAGTTATTTGGG  
TTGTCCATTTCTTAATTTTAAGAGGTGTTAATACAGCTGCATTTATTAATAC  
CGTAGTTACCTTTGCAAAATTAGTACCTGTTATTATTTTCTTAATTTACGCG  
TTATTAGCTTTCAAATTTAACATTTTATGCTTGATATCTGGGGAAATGGAT  
TACATCAATCAATTTCAACCAAGTCAATTCAACTATGAAAACCGCTGTTT  
GGGTATTTATTGGTATTGAGGGCGCCGTTGTCTTCTCAGGTCGTGCTAAAA  
AACACTCTGATATTGGTAAAGCAAGTATCCTAGCATTATTCACATGATTT  
CACTTTATGTATTGATTTCTGTTTTATCACTTGGTATCATGTACGTCCAGA  
ACTTGCAAACTTAAAAACACCAGCTATGGCTTACGTTCTAGAAAAAGCTGT  
TGGTCACTGGGGTGCTATCTTAGTTAACCTTGGTGTTATCATTTACGTATTT  
GGCGCTATTCTTGCTTGGACTTTATTTGCAGCAGAATTACCATATCAAGCT  
GCTAAAGAAGGTGCTTTTCTAAATTTTTTGCAAAAGAAAATAAAAAACA  
AGCTCCAATCAACTCACTCTTAGTCACTAATCTTTGTGTACAAGCATTCTTA  
ATCACGTTCTTATTCACACAAAGTGCTTATCGTTTTGGTTTCGCATTAGCAT  
CATCTGCTATCTTAATTCCTTATGCTTTTACAGCACTATATCAATTACAATT  
CACACTCCGTGAGGATAAGTCAACTCCAGGACATCAAAAGAATTTAATTA  
TCGGTATCCTCGCTACAATCTATGCTGTTTACCTTATCTACGCTGGTGGTTT  
TGATTACTTACTTTTGACAATGATTGCTTATACTCTAGGTATGATTCTCTAT  
ATTAAAATGAGAAAAGATGACAAGCTTGGCGTAATCATGGTCATAGCTGT  
TTCCAGTGTGAAATTGTTATCC

MEKEKKLGLLPLTMLVIGSLIGGGIFDLMQNMSSRAGLVPMLIAWVITAIGMG  
TFVLSFQNLSEKRDLTAGIFS YAKEGFNGFMGFNSAWGYWLSAWLGNVAY  
AALLFSSLGYFFKFFGNGNIIISII GASIVIWVVHFLILRGVN TAAFINTVVTFAK  
LVPVIIFLISALLAFKFNIFSLDIWGNLHQSI FNQVNSTMKTAVWVFIGIEGAV

FIG. 1 CONT'D

40 / 110

VFSGRAKKHSDIGKASILALFTMISLYVLISVLSLGIMSRPELANLKTPAMAYV  
LEKAVGHWGAILVNLGVIISVFGAILAWTLFAAELPYQAAKEGAFPKFFAKEN  
KNKAPINSLLVTNLCVQAFLLITFLTQSAYRFGFALASSAILIPYAFTALYQLQF  
TLREDKSTPGHQKNLIIGILATIYAVYLIYAGGFDYLLLTMIAYTLGMILYIKMR  
KDDKLGVMVIAVSSVKLLS

## Sequence description:

- A] Length: 1356 bp - 452 aa (partial sequence)  
B] ATG start codon is preceded by an possible  
Shine-Dalgarno sequence. Possesses a potential  
leader peptide sequence.

ID-120

Clone 3-8

ATGAAATTTGAAAAACGGCAGGTCTATTATGTTGTCATAACATTTGCTATT  
TGCTATGCTATACAGGCTTATTGGGGAGCTGTTTCTAATATTTTAACTACGC  
TTCATAAGGCAATATTTCTTTTTTGTATGGGAGCTGGAATTGCCTATATTAT  
TAATATTGTAATGTCAGTCTATGAGCGATTATATATAAAGCTTTTTAAAGG  
ATCTAGACTATTAATGGCAATCAAGCGTAGTGTTTCTATGATTTTATCCTAT  
GCAACTTTTATTGGTTTAATTGTCTGGCTATTTTCAATTGTCATTCCAGATT  
TGATTTCTAGTTTGAGTTCTTTATTGGTTATTGATACCGGAGCACTTGCTAA  
ATTGGTTAATAATCTCAATGAAAATAAACAAATTTCTGAGGCTTTAAATTA  
TATGGGAACAGATAAAGACTTAGTTTCTACTTTAAGTGGTTATAGCCAGCA  
GATTTTGAAGCAAGTTTTATCTGTTTTAACAATTTACTAACCTCAGTTTCC  
TCTATTGCGGCAACACTTCTGAATGTTTTGTAGTTTTATTTTTCAATTTA  
CGTTTTGGCAAACAAGGAGCAGTTGGGACGTCAATTTAATTTGTTAATTGA  
TACCTATTTAGGTTCAACAGGCAAAACATTCCATTACGTTTCGTATATCCTT  
CATCAACGTTTCCATGGTTTTTTTGTAAAGCCAACTTTAGAAGCTATGATTT  
TAGGAAGTTTGACGGTTATTGGTATGTTGATCTTCCAATTTCTTATGCTTT  
AACAGTTGGGGTTTTAGTTGCTTTTACAGCTCTAATACCGGTTGTGGGAGC  
CTACATTGGTGTTACAATCGGTTTCATCTTAATTGCTACTGAATCGCTTACT  
GAAGCATTCTTGTTTGTTCTTTTCTTGATCCTTTTACAACAATTTGAGGGAA  
ATGTCATTTATCCGAAAGTTGTCGGTGGATCGATTGGACTGCCTTCTATGT  
GGGTTTTAATGGCTATTACTATCGGAGGTGCTTTATGGGGGATCTTAGGCA

FIG. 1 CONT'D

41 / 110

TGTTACTTGCTGTTCTGTTGCAGCTACTATCTATCAGATTGTAAAAGATCA  
TATTATCAAGCGACAAACGCTTAGAAATCGTGCACGAACCTATCGTTAA

MKFEKRQVYYYVVITFAICYAIQAYWGAVSNILTTLHKAIFPFLMGAGIAYIINI  
VMSVYERLYIKLFKGSRLMAIKRSVSMILSYATFIGLIVWLFSIVIPDLISSLS  
LLVIDTGALAKLVNNLNENKQISEALNYMGTDKDLVSTLSGYSQQILKQVLSV  
LTNLLTSVSSIAATLLNVFVSFIFSIYVLANKQLGRQFNLLIDTYLGSTGKTFH  
YVRHILHQRFHGFFVSQTLEAMILGSLTVIGMLIFQFPYALTVGVLVAFTALIP  
VVGAYIGVTIGFILIA TESL TEAFLFVLFLILLQQFEGNVIYPKVVGGSIGLPSM  
WVLMAITIGGALWGILGMLLAVPVAATIIYQIVKDHIIKRQTLNRARTYR\*

#### Sequence description:

- A] Length: 1134 bp - 378 aa (full-length gene)
- B] ATG start codon is preceded by an typical Shine-Dalgarno sequence. Possesses a potential leader peptide sequence.

ID-121

Identical to ID-68, as described in WO 00/06736

ID-122

Clone 3-16

GTGATTACAATTAAAAAGGAATCTGTTATCAAACCTATTGAAGTATGCTTTT  
GGCATTATAATGGGATTTATTATCTTAGCTATTGTAATAGGTGGGCTCCTA  
TTTGCATACTACGTTAGTCGTTCTCCGAAATTAACCGATCAAGCTTTAAAA  
TCCGTAACTCTAGTTTGGTTTATGATGGTAATAATAAACTTATTGCCGATT  
TAGGCTCAGAAAAGCGTGAAAGTGTTAGTGCGGATAGCATTCCACTAAAT  
TTGGTTAACGCTATCACTTCTATAGAAGATAAACGTTTCTTTAAACATAGA  
GGTGTCGATATTTATCGTATTTTAGGTGCAGCTTGGCATAACCTTGTTAGTA  
GTAATACGCAAGGTGGTTCAACCCTTGATCAACAGTTGATTAACTGGCTT  
ACTTTTCTACCAATAAATCTGACCAAACGTAAAACGTAAATCACAGGAA  
GTTTGGCTTGCGCTTCAAATGGAGCGTAAATACACCAAAGAAGAAATTCTT  
ACTTTCTATATTAATAAAGTTTATATGGGAAATGGGAATTATGGTATGAGA

**FIG. 1** CONT'D

42 / 110

ACAACAGCTAAATCATACTTTGGTAAAGACCTAAAGGAATTATCTATTGCA  
 CAACTTGCTTTGCTCGCTGGTATTCCTCAAGCACCTACACAATATGACCCTT  
 ATAAAAACCCAGAATCTGCTCAAACAAGACGTAATACCGTTCTTCAGCAG  
 ATGTATCAAGATAAAAAACATTTCTAAAAAGGAATACGACCAAGCTGTTGC  
 AACTCCAGTAACTGATGGCTTAAAAGAATTAAAGCAAAAATCTACTTATCC  
 AAAATATATGGATAACTACTTAAAACAAGTTATTAGTGAAGTTAAACAAA  
 AAAGTGGTAAAGATATCTTTACTGCTGGGCTAAAAGTGTATACTAATATCA  
 AACTGATGCACAAAAACAATATATGACATCTACAACAGTGATACTTAC  
 ATCGCTTATCCAAACAATGAATTACAAATAGCATCTACCATCATGGATGCG  
 ACTAATGGTAAAGTCATTGCACAATTAGGCGGGCGTCATCAGAATGAAAA  
 TATTCATTTGGGACAAATCAATCTGTCTTAACAGACCGCGATTGGGGTTC  
 TACAATGAAACCTATCTCAGCTTATGCACCTGCTATTGATAGTGGTGTCTA  
 TAATTCAACAGGTCAATCATTAAACGACTCAGTTTACTACTGGCCTGGTAC  
 TTCTACTCAACTATATGACTGGGATCGTCAATATATGGGTTGGATGAGTAT  
 GCAGACCGCTATTCAACAATCACGTAACGTCCCTGCTGTCAGAGCACTTGA  
 AGCCGCTGGATTAGACGAAGCAAAATCTTTCCTTGAAAAATTAGGCATAT  
 ACTATCCAGAAATG

MITIKKESVIKLLKYAFGIIMGFIILAIVIGLLFAYYVSRSPKLTQALKSVNSS  
 LVYDGNNKLIADLGSEKRESVSADSIPLNLVNAITSIEDKRFFKHARGVDIYRILG  
 AA WHNLVSSNTQGGSTLDQQLIKLAYFSTNKSQTLKRKSQEVWLALQMER  
 KYTKEEILTFYINKVYMGNGNYGMRTTAKSYFGKDLKELSIAQLALLAGIPQA  
 PTQYDPYKNPESAQTRRNTVLQQMYQDKNISKKEYDQAVATPVTGDLKELK  
 QKSTYPKYMDNYLKQVISEVKQKTGKDIFTAGLKVYTNINTDAQKQLYDIYN  
 SDTYIAYPNNELQIASTIMDATNGKVIAQLGGRHQNENISFGTNQSVLTDRDW  
 GSTMKPISAYAPAIIDSGVYNSTGQSLNDSVYYWPGTSTQLYDWRQYMGWM  
 SMQTAIQSRNVPVRALEAAGLDEAKSFLEKLGIIYPEM

## Sequence description:

- A] Length: 1386 bp - 462 aa (partial sequence)
- B] GTG start codon is preceded by an  
 typical Shine-Dalgarno sequence. Possesses a  
 potential leader peptide sequence.

ID-123

Clone 3-17

FIG. 1<sub>CONT'D</sub>

43 / 110

ATGGCTAATGTATATGATTTAGCAAATGAATTAGAACGTGCTGTTTCGTGCT  
TTACCAGAATACCAAGCAGTTTAACTGCAAAAGCAGCTATTGAAAATGA  
TGCGGATGCACAAGTGCTTTGGCAAGACTTTTTGGCTACCCAATCAAAAGT  
TCAAGAAATGATGCAATCTGGCCAAATGCCAAGTCAAGAAGAACAAGATG  
AAATGTCTAAACTTGGGGAAAAAATTGAATCCAATGACCTTTTAAAAGTTT  
ATTTTGACCAACAACAACGGTTGTCTGTCTATATGTCTGATATCGAAAAAA  
TTGTCTTTGCACCCATGCAGGACTTGATGTAA

MANVYDLANELERAVRALPEYQAVLTAKAAIENDADAQVLWQDFLATQSK  
VQEMMQSGQMPSQEEQDEMSKLGEKIESNDLLKVYFDQQRLSVYMSDIEKI  
VFAPMQDLM\*

Sequence description:

- A] Length: 336 bp - 112 aa (full length sequence)
- B] ATG start codon is preceded by an  
typical Shine-Dalgarno sequence. No obvious  
potential leader peptide sequence.

ID-124

Clone 3-26

ATGGCAGAAATCACAGCTAAACTTGTAAGAATTGCGTGAAAAATCAGG  
TGCAGGCGTTATGGACGCTAAAAAAGCATTAGTAGAACTGATGGTGACC  
TTGATAAAGCGATTGAATTACTTCGCGAAAAAGGTATGGCTAAAGCAGCT  
AAAAAAGCAGACCGTGTTGCTGCTGAAGGTTTAACAGGTGTTTATGTTGAT  
GGTAACGTTGCAGCAGTTATTGAAGTTAA

MAEITAKLVKELREKSGAGVMDAKKALVETDGDLDKAIELLREKGMAKAAK  
KADRVAEGLTGVYVDGNVAIVIEV

Sequence description:

- A] Length: 230 bp - 76 aa (partial sequence)
- B] ATG start codon is preceded by an  
typical Shine-Dalgarno sequence. No obvious  
potential leader peptide sequence.

FIG. 1 CONT'D

44 / 110

ID-125

Clone 3-33

ATGATAAAAAACCTGTTATTAACAGGTTTTTTATCATTTAATGACGGAAAA  
CTGGACACAAATTATTTTTCTTGTATAATTAAATATATTATTTCTTATCAGG  
AGGTTATGATGACATTAGAGAAACGATTAA

MIKNLLLTGFLSFNDGKLDTNFYFSCIIFYIISYQEVMMTLEKRF

Sequence description:

- A] Length: 134 bp - 44 aa (partial sequence)
- B] ATG start codon is preceded by an  
typical Shine-Dalgarno sequence. Possible  
potential leader peptide sequence.

ID-126

Clone 3-41

ATGAAAAATAATAAAAAATAATGGTTTTCTGAAAAATTCCTTTATTTACATA  
TTATTGATTATTGCGGTTATTACAACCTTCAATACTATTAA

MKNNKNNGFLKNSFIYILLIIVITTFQYYL

Sequence description:

- A] Length: 94 bp - 31 aa (partial sequence)
- B] ATG start codon is preceded by a  
possible Shine-Dalgarno sequence. Potential  
leader peptide sequence.

**FIG. 1** CONT'D

45 / 110

ID-127

Clone 3-42

ATGTTAGATATTATCTTATCCGGAATTTGCAAGGATTACTTTGGTCAATTA  
TGGCAATTGGCGTGTTTATCACTTTTCGTATCTTAGACATAGCCGATCTCTC  
TGCAGAAGGGGCTTCCCTATGGGGGCTGCAGTTTGCGCCTTATGTATCGT  
TAA

MLDIILSGISQGLLWSIMAIGVFITFRILDIADLSAEGAFPMGAAVCAIV

Sequence description:

- A] Length: 158 bp - 52 aa (partial sequence)
- B] ATG start codon is preceded by a possible Shine-Dalgarno sequence. Potential leader peptide sequence.

ID-128

Clone 3-43

ATGGAAATGCCTAAAAGAAATGAATTACTCAATAAAGAAATTAAAATGAG  
TATTGATAAACTTAGATATAAAGAACCAGAGAGTGAACATGACAAGCGAC  
CTACTTTTTATTTGGTAGTACTTATACTTGTTACTGTAGCAGTTATATTGTC  
GTTATTTAA

MEMPKRNEILLNKEIKMSIDKLRYKEPESEHDKRPTFYLVVLILVTVAVILSLF

Sequence description:

- A] Length: 161 bp - 53 aa (full-length gene)
- B] ATG start codon is preceded by a possible Shine-Dalgarno sequence. Potential

**FIG. 1** CONT'D

46 / 110

leader peptide sequence.

ID-129

Clone 3-44

GTGGTAAGTAAATTGAGTTTAAACAACGATTTTTGCATTGCTATTTTCATCA  
ATGCTAATTTACGCAACACCTCTTATCTTTACAAGTATTGGGGGAACCTTC  
TCTGAACGTGGTGGTATCGTCAACGTTGGTTTAGAAGGAATTATGGTAATT  
GGAGCTTCTCAGGCGTTGTATTAA

MVSKLSLTTIFALLFSSMLIYATPLIFTSIGGTFSEGGIVNVGLEGIMVIGAFSG  
VVF

Sequence description:

- A] Length: 179 bp - 59 aa (partial sequence)
- B] GTG start codon is preceded by a possible Shine-Dalgarno sequence. Potential leader peptide sequence.

ID-130

Clone 3-46/47

ATGAGAATTATTGCAATAACTGAAAAGGTTATAAAAGAACTGTTTCGTGAT  
AAAAGAACACTTGCTATGATGTTTTTAGCACCTATTTAATTATGTTTTTGA  
TGAATGTTATGTTTTCTGCGAATAGTAATACAAAAGTTAAGATTGGAAC  
TTAACGTTAACACGAAGGTCGTTTCAAATTTAGATAATATTAAGCATATTC  
AAGTGAGATCATTTAAATTTAACTCATCTGCTAAAAAGCACTCAAATCAA  
ATAAAATTGATGCTCTTATTTTCGGAGGACAATAAATCTTATACTGTCTTCT  
ATGCGAATACAGATTCTTCAAAGACGACTTTAACAAGACAAGCTTTTAA  
ACCGCTGTTAATAACAATGAACAGTAAGGAACTGATTTTCGCAAGTTAAAT  
TTAGCTAATAAGAATCCGAACTAGCACAATCCTTACAACTCGCTCCAAA  
TATATCAAAGAAAAATATAATTACGGAAATAAAAAATACAGGCTTTTTTGC  
AAAAATGATACCAATACTAATGGGATTTATGGTCTTCTTCTTGGTTTTT

FIG. 1 CONT'D

47 / 110

MRIIAITEKVIKELFRDKRTLAMMFLAPILIMFLMNVMFSA NSNTKVKIGTINV  
NTKVVS NLDNIKH IQVRSFKFNSSAKKALKSNKIDALISEDNKS YTVFYANTDS  
SKTTLTRQAFKTA VNTMNSKELISQVKILANKNP KLAQSLQTRSKYIKEK YNY  
GNKNTGFFAKMIPILMGFMVFFLVF

## Sequence description:

- A] Length: 558 bp - 186 aa (partial sequence)
- B] ATG start codon is preceded by a possible Shine-Dalgarno sequence. Potential leader peptide sequence. C-terminus has yet to be determined.

ID-131

Clone 3-48

GTGATTATCGTTATGAGTAAACATCAAGAAATTTTGGAGTACCTAGAAAAT  
TTAGCTGTTGGTAAGAGGGTTAGTGTACGCAGTATTTC AAATCATT TAA

MIIVMSKHQEILEYLENLAVGKRVS VRSISNHL

## Sequence description:

- A] Length: 100 bp - 33 aa (partial sequence)
- B] GTG start codon is not preceded by a obvious Shine-Dalgarno sequence. No obvious leader peptide sequence.

ID-132

Clone 2-c53

FIG. 1 CONT'D

48 / 110

ATGTATAGAGAAATTACCGCTGTCGAACACGATCGCTTTGTGAGCGAATCC  
AACCAAACAAACCTACTTCAATCTCTTAATTGGCCCAAAGTAAAAGACAA  
CTGGGGTAGTCAATTACTTGGCTTTTTTTGACGGTGAAACCCAAATTGCCAG  
CGCTAGTATTCTCATCAAATCACTTCCTCTTGGCTTCTCCATGCTGTATATT  
CCGCGTGGACCAATCATGGATTACTCCAATCTAGATATTGTAACCTAAGGTC  
CTTAAGGACCTTAAAGCTTTTGGCAAAAAACAAAGAGCTCTCTTTATCAAG  
TGTGATCCTCTCATCTATTT

MYREITAVEHDFVSESNQTNLLQSLNWPVKDNWGSQLLGFFDGETQIASA  
SILIKSLPLGFSMLYIPRGPIMDYSNLDIVTKVLKDLKAFGKKQRALFIKCDPLI  
Y

Sequence description:

- A] Length: 326 bp - 108 aa (partial sequence)
- B] ATG start codon is preceded by an obvious Shine-Dalgarno sequence. No obvious leader peptide sequence.

ID-133

Clone 2-c59

ATGGACAAGAAAAAATCTTAGTAACGGGTATTGTGCCTAAAGAAGGTCT  
AAGAAAGCTTATGGACCGATTTGATGTTACTTATTCAGAAGATCGCCCAT  
TTCACGTGACTATGTGTTAGAGCATTTATCTGAATATGACGGATGGTTACT  
CATGGGACAAAAAGGTGATAAAGAGATGATTGATGCAGGTGAAAACCTAC  
AAATTATTTCTTT

MDKKKILVTGIVPKEGLRKLMDRFDVTYSEDRPFSRDYVLEHLSEYDGWLLM  
GQKGDKEMIDAGENLQIIS

Sequence description:

- A] Length: 215 bp - 71 aa (partial sequence)

**FIG. 1** CONT'D

49 / 110

B] ATG start codon is preceded by an obvious Shine-Dalgarno sequence. No obvious leader peptide sequence.

ID-134

Clone 2-c62

ATTTCGAAAGATGACTACCAAAATATTAGTTTTGGACAGGATCCAGAAGTT  
GTTGATTATGCTGGTCTGTTTGAAAAACGCCGTCCAGTTTTAGAAAAAGCA  
GTTAAAAATTTCTTGCAAGAAGAGAGAGCTACGAGAATGCTATCTGATTTC  
TTGCAAGAAGAAAAATGGGTAAGTGAATTTGCTGAATTTATGGCGATCAA  
AGAACATTTTGGTAATAAGGCGCTTCAAGAATGGGATGACAAGGCTATTA  
TACGCCGCGAAGAAGAAGCCTTAGCAGGATATCGTCAAAAGCTTAGTGAA  
GTGATAAAATATCATGAAGTAACGCAATATTTCTTTTACAAACAATGGTTT  
GAGTTAAAAGAATATGCTAATGATAAAGGGATTCAAATTATCGGTGATAT  
GCCAATCTACGTTTCTGCCGATAGTGTAGAAGTTTGGACAATGCCTGAACT  
GTTT

ISKDDYQNISFGQDPEVVDYAGLFEKRRPVLEKAVKNFLQEERATRMLSDFLQ  
EEKWVTDFAEFMAIKEHFGNKALQEWDDKAIIRREEEALAGYRQKLSEVIKY  
HEVTQYFFYKQWFELKEYANDKGIQIIGDMPIYVSADSVEVWTMPFLF

A] Length: 459 bp - 153 aa (partial sequence)  
B] More sequencing is required to determine the  
N- and C-termini  
enzyme). - *Streptococcus pneumoniae* (63%)

ID-135

Identical to ID-108 described in WO 00/06736

Clone 2-c63

ID-136

Clone 2-c66

FIG. 1<sub>CONT'D</sub>

50 / 110

ATGGCAAAACAGAAAAATAACTGGCGCCGTGTTGGAGTTGGTGTCTTAC  
 ACTTGCTTCAGTTGCGACTCTTGCTGCATGTGGAAGTAAATCAGCTTCCCA  
 GGATTCTAATGGAGCGATTAATTGGGCTATTCCAACAGAAATCAATACACT  
 AGATTTATCTAAAGTTACAGACACTTACTCAAATCTAGCTATTGGTAACTC  
 TAGTAGTAATTTCTTCGCTTAGATAAAGATGGAAAGACAAGACCAGACTT  
 GGCTACTAAAGTTGATGTTTCAAAGATGGCTTAACTTATACAGCTACATT  
 ACGTAAAGGCTTGAAGTGGTCAGATGGCAGTAACTTACTGCAAAGGATT  
 TTGTTTATTCATGGCAACGTTTAGTTGATCCTAAAACAGCTTCACAATATG  
 CTTACCTTGCTGTTGAAGGGCATGTGCTTAATGCCGATAAAATCAACGAAG  
 GACAAGAGAAAGACTTGAATAAGCTAGGTGTTAAGGCAGAAGGCGATGA  
 CAAAGTTGTTATTACTTTATCTAGTCCGTCTCCGCAATTCATCTACTACCTT  
 GCATTCACTAACTTCATGCCACAAAAACAAGAAGTTGTTGAAAAATATGG  
 AAAAGATTACGCAACTACTTCAAAAAATACAGTTTACTCAGGACCATATA  
 CTGTTGAAGGTTGGAATGGTTCGAATGGTACTTTCACGCTGAAGAAAAAC  
 AAAAATTATTGGGACGCTAAAAATGTAAAAACAAAAGAAGTTCGCATCCA  
 GACTGTAAAAAAACCAGATACCGCCGTTCAAATGTATAAACGTGGTGAGT  
 TAGATGCAGCTAATATCTCAAATACTTCTGCTATTTATCAAGCTAATAAAA  
 ATAATAAGATGTCACAGATGTTCTAGAAGCGACCACTGCCTATATGGAA  
 TATAATACTACTGGTTCTGTGAAAGGGCTTGATAATGTTAAGATTTCGTGCG  
 GCCTTAACTTAGCAACTAACCGTAAAGGAGTTGTTCAAGCAGCCGTTGAT  
 ACAGGCTCAAACCGGCAATTGCTTTTGCACCTACTGGTTTAGCCAAAACA  
 CCAGATGGAAGTGAATTTGGCAAAATATGTTGCCCCAGGTTATGAATATAAT  
 AAAACTGAAGCAGCAAACTCTTTAGACTA

MAKQKNNWRRVGVGVLTLASVATLAACGSKSASQDSNGAINWAIPTEINTLD  
 LSKVTDITYSNLAIGNSSSNFLRLDKDGKTRPDLATKVDVSKDGLTYTATLRKG  
 LKWSDGSKLTAKDFVYSWQRLVDPKTASQYAYLAVEGHVLNADKINEGQEK  
 DLNKLGVKAEGDDKVVITLSSPSQFIYYLAFTNFMPPQKQEVVEKYGKDYAT  
 TSKNTVYSGPYTVEGWNGSNGTFTLKKKNKNYWDAKNVKTKEVRIQTVKKPD  
 TAVQMYKRGELDAANISNTSAIYQANKNNKDVTDVLEATTAYMEYNTTGSV  
 KGLDNVKIRRALNLATNRKGVVQAAVDTGSKPAIAFAPTGLAKTPDGTDLAK  
 YVAPGYEYNKTEAAKLFRL

Sequence description:

- A] Length: 1143 bp - 381 aa (partial sequence)
  - B] Shine-Dalgarno sequence precedes ATG codon.
- Possesses a potential leader peptide sequence.

FIG. 1<sup>CONT'D</sup>

51 / 110

ID-137

Clone 2-c67

TTGAGAGTTTATGAAAATAAAGAAGAGTTGAAAAAAGAAATAAGTAAAAC  
ATTTGAGAAATACATTATGGAATTTAATAA  
TATTCCAGAGAATCTAAAAGATAAAAGAATTGATGAAGTTGATAGAAGTC  
CAGCAGAAAACCTTTCTTATCAGGTTGGCT  
GGACCAACTTGGTTCTTAAATGGGAAGAAGATGAAAGAAAGGGACTTCAA  
GTAAAAACACCATCGGATAAATTT

MRVYENKEELKKEISKTFEKYIMEFNNIPENLKDKRIDEVDRTPAENLSYQVG  
WTNLVLKWEEDERKGLQVKTPSDKF

## Sequence description

- A] Length: 234 bp - 78 aa (partial sequence)
- B] TTG start codon is preceded by a potential Shine-Dalgarno sequence. No obvious leader peptide sequence.

ID-138

Clone 2-c70

ATGTCAAAGTTTGATAGTCAGAAAATAATTACTCCGATTATGAAGTTTGTC  
AATATGCGAGGGATTATTGCACTCAAAGATGGCATGCTAGCAATTTTACCA  
CTAACAGTTGTTGGGAGTCTCTTTTAAATATTAGGGCAGCTTCCATTT

MSKFDSQKIITPIMKFVNMRGIIALKDGMLAILPLTVVGSFLILGQLPF

## Sequence description

- A] Length: 150 bp - 50 aa (partial sequence)
- B] ATG start codon is preceded by a potential Shine-Dalgarno sequence. Possesses a potential

FIG. 1 CONT'D

52 / 110

leader peptide sequence.

ID-139

Clone 2-c71

GAGACCACTTCATCAGTTAAACCAGCAGGAATTGACCGTATCAATCATACC  
TCAACACCCCCGAAGAAAACACTACCCCCAACATTGCAACGACGCATAGCTT  
CAAAGATCGTTGTGATACTTTAGAAAGAATTCACAATGAAGACATTGATGT  
TTGTTCTGGATTCAATTTGTGGTATGGGAGAGAGCGATGAGGGGCTCATCAC  
ATTAGCTTTCAGACTAAAAGAACTGAACCCCTATTCTATCCCTGTCAATTTT  
TACTTGCTGTTGAAGGAACACCTCTTGGAATAATACTATTTGACTCCC  
ATTAAATGCTTAAAAATTATGGCCATGTTGCGTTTTGTTTTTCCTTTCAAGG  
AATTAAGATTAAGTGCTGGACGGGAGGTCCATTTTGAGAATTTTGAATCAT  
TAGTCACCTTACTTGTGACTCAACTTTTTTGGGAAATTACCTAACAGAGG  
GGGGTCGCAATCAACATACCGATATTGAATTCTTGGAATAATTACAATA  
AATCATACTAAAAAGGAATTAATTT

ETTSSVKPAGIDRINHTSTPPKKTTPNIAATTHSFKDRCDTLERIHNEIDVCSGFI  
CGMGESDEGLITLAFRLKELNPYSIPVNFLLAVEGTPLGKYNLTPIKCLKIMA  
MLRFVFPFKELRLSAGREVFHFNESLVTLVDSTFLGNYLTEGGRNQHTDIEF  
LEKLQLNHTKKELI

Sequence description:

A] Length: 535 bp - 178 aa (partial sequence)  
B] N- and C-termini require verification

ID-140

Clone 2-c73

ATGCCGGTTTGGACTGCACAGTCTATTCCAAAGGCATTTTGTAGAAAAGCAT  
AATACTAAGGAAGGCACCTGGGCAAACTAACCATTCTAAGTGGTTCTTTA  
GTATTTTACCAGTTATCTCCTGATGGAGAGGAAATCTCGCGGCATATTTT

**FIG. 1** CONT'D

53 / 110

GATGCTAGTAGTGATATTCCTTTTGTGATCCACAAGTCTGGCATAAAGTT  
TCGCCGAATAGTCCAGACTTAAGTTGCTATCTAACTTTTTACTGCCAAAAA  
GAAGATTACTTCCATAAAAAAATATGGTCTCACGCGCACACATTCTGAGGTT  
ATCGCCAGTGCACCTCTCTTATCTGAGAAGAGTAATATATTAGACCTTGGG  
TGTGGTCAAGGGCGAAACTCACTTTATTTATCGCTGCTGGGACATCAAGTG  
ACTTCTGTGCGATTCAAACGGACAGAGCCTTGTAGCTTTAGAAAATATGGCA  
TTAGAAGAAGAGCTTCCTTACAATATAAAAAGGTATGATATTAATACTACT  
GCTATTGAAGGGCACTATGATTTTATTTTATCAACTGTGGTATTTATGTTTT  
T

MPVWTAQSIPKAFLEKHNTKEGTWAKLTILSGSLVIFYQLSPDGEEISRHIFDAS  
SDIPFVDPQVWHKVSPNSPDLSCYLTIFYCQKEDYFHKKYGLTRTHSEVIASAP  
LLSEKSNILDLGCGQGRNSLYLSLLGHQVTSVDSNGQSLVALENMALEEELPY  
NIKRYDINTTAIEGHYDFILSTVVFMF

Sequence description:

A] Length: 563 bp - 187 aa (partial sequence)  
B] N- and C-termini require verification

ID-141

Clone 2c76

ATGACAAAGCAAATAATTGCCATTTGGGCTGAAGATGAAGACCATTTGAT  
TGGAGTTAATGGCGGTTTACCATGGAGGCTTCCTAAAGAGTTACATCACTT  
CAAAGAAACGACCATGGGGCAGGCTTTGCTTATGGGACGAAAGACCTTTG  
ATGGAATGAACCGTCGTGTTTTACCTGGTAGAGAGACAATCATCTTAACAA  
AAGATGAACAATTCCAAGCAGATGGAGTGACAGTCCTAAATAGTGTTGAA  
CAAGTTATAAAATGGTTTCAGGAACATAATAAGACCTTATTTATTGTAGGT  
GGTGCAAGTATTTATAAAGCATTTCTGCCTTATTGTGAAGCAATCATAAAA  
ACTAAAGTTCATGGAAAATTCAAAGGTGATACCTATTTTCCTGATGTTAAT  
CTATCTGAGTTT

MTKQIIAIWAEDEDHLIGVNGGLPWRLPKELHHFKETTMGQALLMGRKTFDG  
MNRRLVPGRETIILTKDEQFQADGVTVLNSVEQVIKWFQEHNKTLFIVGGASI  
YKAFLPYCEAIKTKVHGKFKGDTYFPDVLNLFSEF

FIG. 1<sub>CONT'D</sub>

54 / 110

## Sequence description:

- A] Length: 417 bp - 139 aa (partial sequence)  
B] ATG start codon is preceded by a Shine-Dalgarno sequence. No leader peptide sequence

ID-142

Clone 2-c78

TTGTGGCCAACTGTGCCCCGCTTATTAATAGCACTTTGTTCCACCATTGAA  
GATATCTTAACATCAGGTGCTCATAGCAACCCTATTTTAATGGGGGTTATA  
CTTGGCGGGACAATTGTAGTAGTGGCGACAGCACCACCTTTCTTCTATGGCA  
TTGACAGCTATGCTAGGATTAACCGGAATGCCTATGGCTATAGGAGCCTTG  
TCTGTCTTTGGTTCGTCATTTATGAATGGTGTACTTTTCCATAAATTAAC  
TTGGAAGTCGTAAAGATAATATAGCTTTTGCTGTTGAGCCTCTAACTCAAG  
CTGACGTGACTTCAGCTAACCCTATTCCAATCTATGTCACTAATTTGTTGG  
TGGTGCAGCTTGTGGTATTTTAATTGCCTTGATGAAATTAGTTAATGATACT  
CCTGGAACAGCGACACCAATTGCAGGATTTGCTGTCATGTTTGCCTATAAC  
CCAATGATAAAAGTACTAATAACCGCTCTAGGTTGTATTATCCTATCTTTA  
CTAGCAGGCTATTTTGGAGGCATTGTTTTT

MWPNCAPLINSTLFTIEDILTSGAHSNPILMGVILGGTIVVVATAPLSSMALTA  
MLGLTGMPMAIGALSVFGSSFMNGVLFHKLKLGSRKDNIAFAVEPLTQADVT  
SANPIPIYVTNFBVGGAAACGILIALMKLVNDTPGTATPIAGFAVMFAYNPMIKVL  
ITALGCILSLLAGYFGGIVF

## Sequence description:

- A] Length: 540 bp - 180 aa (partial sequence)  
B] N- and C-termini have yet to be elucidated

ID-143

FIG. 1 CONT'D

55 / 110

Clone 2-c80

ATGTTTTTAAGTATAATGGCAGGTGTCATAGCATTTGTCCTGACAGTTATT  
GCCATTCCACGCTTCATTAAGTTTTACCAATTGAAGAAAATTGGCGGGCAA  
CAAATGCATGAAGATGTCAAACAACATCTAGCCAAAGCAGGTACGCCGAC  
AATGGGAGGAACGGTATTTT

MFLSIMAGVIAFVLTVIAIPRFIKFYQLKKIGGQQMHEDVKQHLAKAGTPTMG  
GTVF

Sequence description:

- A] Length: 172 bp - 57 aa (partial sequence)
- B] Shine Dalgarno sequence precedes 'ATG' start codon. Possesses a potential leader peptide sequence.

ID-144

Clone 3-83

ATGAAACCATATTTATCTTTTATTGGTAGAACGTTATTATACTTCGGTATTT  
TATTGTTACTAATTTACTTTTTTGCATACCTTGGTCGCGGACAAGGCAGTTT  
TATTTATAA

MKPYLSFIGRTLTYFGILLLLIYFFAYLGRGQGSFIY

Sequence description:

- A] Length: 113 bp - 37 aa (partial sequence)
  - B] Putative ATG start codon is preceded by a typical Shine-Dalgarno sequence. Possesses a potential leader peptide sequence.
- This orf is not in frame with nuc

**FIG. 1** CONT'D

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ID-145

Clone 3-86

ATGTCATATTTTAGAAATTACTGGTATCGTTTTGGAGCAATTTTATTTATTA  
TTTTAGCAGTAATATTGCTTGTTTTTAGACCTGACTGGTCAATGCTTCACTA  
TCTATTGTATTTTACTTTATGGCACTTCTAGCGCATCAATTTGAAGAATAT  
CAGTTTCCCGGTGGGGCATCACCTATCATTAACCTATGTTGTTTATGATGAA  
GAAGAGCTGATGGATTGTTTTCCAGGCAATACTCAGTCTATTATGTTGGTT  
AATACTATTGCTTGGTTGCTTTACATTGCTAGTATTGCTTTTCCTCAAGCTT  
ATTGGCTTGGATTAGGAGTCATGTTCTTTAGTCTAACGCAGCTCTTGGGTC  
ATGGTTTTTCAGATGAATATTAACCTTAAACTTGGTATAATCCTGGTCTAG  
CAACGACAGTATTTCTCCTAGTACCAATAGCTTGCGCATACATCTATCAAG  
CTAGTGCAGAAGGAATGCTCACTTGGGGAGATTGGCTAGGTGGTTTTATCA  
TGTTGATTGTCTGTGTACTAACTAGCATTATTGCACCTGTACAGCTATTGAA  
GGATAAGGAGACCAATTATATTATTAGTCCTTGGCAAATGGACCGTTTTCA  
TAAGGTCGTTAATTTTGTAAGGATAAAAAAATAA

MSYFRNYWYRFGAILFIILAVILLVFRPDWSMLHYLLYFYFMALLAHQFEEYQ  
FPGGASPIINYVVYDEEELMDCFPNGTQSIMLVNTIAWLLYIASIAFPQAYWLG  
LGVMFFSLTQLLGHGFQMNILKKTWYNPGLATTVFLVPIACAYIYQASAEG  
MLTWGDWLGGFIMLIVCVLTSIIAPVQLLKDKETNYIISPWQMDRFHKVVNFV  
RIKK\*

Sequence description:

- A] Length: 651 bp - 219 aa (full length gene)
- B] Putative ATG start codon is preceded by a typical Shine-Dalgarno sequence. Possesses a potential leader peptide sequence.

ID-146

Clone 3-c88

FIG. 1<sub>CONT'D</sub>

57 / 110

ATGCCACTTACAGCACTTGAAATTAAAGATAAAACATTTTCATCAAAATTT  
CGCGGTTATAGCGAAGAAGAAGTT

MPLTALEIKDKTFSSKFRGYSEEEV

Sequence description:

- A] Length: 75 bp - 25 aa (partial sequence)
- B] Putative ATG start codon is preceded by a typical Shine-Dalgarno sequence. No leader peptide

ID-147

Clone 3-90

ATGTCACCTTTTTCAAGAAAAAATTGCTTACAATTGCGCTAAAAAGGAAGCG  
CTTTATAAAGAGAGTTTAGGACGCTACGCCTTGAGATCAATGCTAGCAGG  
GGCTTATTTGACAATGAGTACTGCTGCCGGTATCGTCGCAGCTGATACTAT  
TGGTAAAATTTCTCCTGCTCTATCAGGTTTTGTATTTGCTTTCATCTTTAGTT  
TTGGACTTATTTATGTTTTAATATTTAATGGTGAATTGGCGACATCTAATAT  
GCTTTATCTCACTGCAGGAGCCTATAATAAAAATATCTCTTGGAAAAAAGC  
CATAACAATTTTAATTTATTGTACTTTTTTCAACCTCGTTGGTGCTTGTATA  
TTAGCTTGGTTGTTTAA

MSLFQEKIAYNCAKKEALYKESLGRYALRSMLAGAYLTMSTAAGIVAADTIG  
KISPALSGFVFAFIFSFGLIYVLIFNGELATSNMLYLTAGAYNKNISWKKAITILI  
YCTFFNLVGACILAWLF

Sequence description

- A] Length: 406 bp - 125 aa (partial sequence)
- B] Putative ATG start codon is preceded by a typical Shine-Dalgarno sequence. Possible leader peptide

FIG. 1 CONT'D

58 / 110

ID-148

Clone 3-92

AAGTTACAAGCGACTGAAGTTAAGAGCGTTCCGGTAGCACAACCAGCTTC  
AACAAACAATGCAGTAGCTGCACATCCTGAAAATGCAGGGCTCCAACCTC  
ATGTTGCAGCTTATAAAGAAAAAGTAGCGTCAACTTATGGAGTTAATGAA  
TTCAGTACATAACCGTGCGGGAGATCCAGGTGATCATGGTAAAGGTTTAGC  
AGTTGACTTTATTGTAGGTAAAAACCAAGCACTTGGTAATGAAGTTGCACA  
GTACTCTACACAAAATATGGCAGCAAATAACATTTTCATATGTTATCTGGCA  
ACAAAAGTTTTATTCAAATACAAATAGTATTTATGGACCTGCTAATACTTG  
GAATGCAATGCCAGATCGTGGTGGCGTTACTGCCAACCCTATGACCACGT  
TCACGTATCATTTAA

KLQATEVKSPVPAQPASTTNAAHPENAGLQPHVAAYKEKVASTYGVNEF  
STYRAGDPGDHGKGLAVDFIVGKNQALGNEVAQYSTQNMAANNISYVIWQQ  
KFYSNTNSIYGPANTWNAMPDRGGVTANHYDHSVHVSF

Sequence description

- A] Length: 419 bp - 139 aa (partial sequence)  
B] N- and C-termini have yet to be determined

ID-149

Clone 3-94

ATGATTCCAGTAGTTATTGAACAAACAAGTCGTGGTGAACGTTCTTATGAT  
ATTTACTCACGTCTTTTAAAAGATCGTATTATTATGTTGACAGGCCAAGTT  
GAGGATAATATGGCCAATAGTATCATTGCACAGTTATTGTTTCTCGATGCA  
CAAGATAATACAAAGGATATTTACCTTTATGTCAATACACCAGGTGGTTCA  
GTATCGGCTGGACTTGCTATTGTGGACACCATGAACCTTCATTAAATCGGAC  
GTACAGACGATTGTTATGGGGATGGCTGCTTCGATGGGAACCATTATTGCT  
TCAAGTGGTGCTAAAGGAAAACGTTTTATGTTACCGAATGCAGAATATATG

FIG. 1 CONT'D

59 / 110

ATCCACCAACCAATGGGCGGAACAGGCGGAGGTACACAGCAATCTGATAT  
 GGCTATCGCTGCTGAGCATCTTTTAAAAACGCGTCATACTTTAGAAAAAAT  
 CTTAGCTGATAATTCTGGTCAATCTATTGAAAAAGTCCATGATGATGCAGA  
 GCGTGATCGTTGGATGAGTGCTCAAGAACACTTGATTATGGCTTTATTGAT  
 GCTATTATGGAAAATAATAATTTACAATAATAGATTTAAAAGAGTTGAGTT  
 TACCAACTCTTTTTTTATTTGTTGGAATTATGTTATAATCTTAGTAATTACA  
 GATATGACGCAGAAAGGAAAAAATTATTGA

MIPVVIEQTSRGRERSYDIYSRLLKDRIIMLTGQVEDNMANSIIAQLLFLDAQDN  
 TKDIYLYVNTPPGGSVSAGLAIVDTMNFIKSDVQTIVMGMAASMGTHASSGAK  
 GKRFMLPNAEYMIHQPMGGTGGGTQQSDMAIAAEHLLKTRHTLEKILADNSG  
 QSIEKVHDDAERDRWMSAQEHLIMALLMLLWKIIIYNNRFRVEFTNSFFICW  
 NYVIILVITDMTQKGKNY\*

#### Sequence description

- A] Length: 693 bp - 231 aa (full length gene)
- B] Putative ATG start codon is preceded by a typical Shine-Dalgarno sequence. No leader peptide. Significantly, it would appear to have a very hydrophobic C-terminus.

ID-150

Clone 2-c86

ATGAAACCAAAAATTATTGGTGTACTTGGTCTAGGAATATTTGGACAAACA  
 CTCGCACAAGAACTAAGTAACCTTTGAACAAGATGTTATTGCTATTGACAGC  
 AATCCTGAAAATGTACAAGCTGTCGCCGAAGT  
 TGTTACAAAAGCAGCTATCGGAGACATTACTGATTTAGCTTTCCTAAAACA  
 CATCGGGATCAGTGACTGTGATACTGTTATTATTGCTACAGGAAACAGTTT  
 AGAGAGCTCAGTATTGGCCGTAATGCACTGTAAAAAGTTAGGCGTCCCAC  
 AAGTTATTGCTAAAGCTCGAAACCTTGTATACGAAGAAGTACTTTATGAAA  
 TTGGTGCTGATTTGGTTATCTCTCCGGAGCGAGAATCTGGGCAAAATGTTG  
 CTGCAAACCTCATGAGAAATAAAATTACAGATGTCTTCCAGATTGAATCTG  
 ATATTTCTGTCAATTGAATTT

MKPKIIGVLGLGIFGQTLAQELSNFEQDVIAIDSNPENVQAVAEVVTKAAIGDI  
 TDLAFLKHIGISDCDTVIIATGNSLE

## FIG. 1<sub>CONT'D</sub>

60 / 110

SSVLAVMHCKKLGVPQVIKARNLVYEEVLVEIGADLVISPERESGQNVAAN  
LMRNKITDVFQIESDISVIEF

## Sequence description:

- A] Length: 459 bp - 153 aa (partial sequence)
  - B] Putative ATG start codon is preceded by a typical Shine-Dalgarno sequence. Possesses a potential leader peptide sequence.
- This orf is not in frame with nuc

ID-151

Clone 2-c88

GTGCGTTATAGTAAAGAGATTATTCAGTTAGCTATACCAGCTATGATTGAA  
AATATCTTACAAATGCTCATGGGAGTAGTTGATAATTATCTAGTGGCTCAG  
TTAGGTGTTGTAGCAGTATCAGGTGTTTCAGTTGCTAATAATATAATTACT  
ATTTATCAAGCTATTTTTATAGCTTTAGGGGCGAGTATAGCAAGTCTATTG  
GCCAAGTCGTTAGCAGGTAGTGAGAAGGATGATGCAATTTTCAGTATGTTCT  
CAAGCCATTTTTCTAACATCACTGATAGGGGCAGTATTAGGAATTATCTCG  
ATTGTTTTTGGACAAACTTTCTTT

MRYSKEIIQLAIPAMIENILQMLMGVVDNYLVAQLGVVAVSGVSVANNIITIY  
QAIFIALGASIASLLAKSLAGSEKDDAISVCSQAIFLTSLIGAVLGIISIVFGQTF

## Sequence description

- A] Length: 330 bp - 110 aa (partial sequence)
- B] Putative GTG start codon is preceded by a typical Shine-Dalgarno sequence. May have a leader peptide

ID-152

FIG. 1<sub>CONT'D</sub>

61 / 110

## Clone 2-c92

TTGATTAACAAGTATTCGTGCTTTTTGAAGAGGATTCTCCATAATAATACT  
CCTTTAATAGTTATCGTGAGAAGTATTTTAAAGAAAAACCGCCAAGGTAG  
AGCGACATTTCTGCCTTTAACTACAATAAAACCAAGAGAATTAGCACAAAC  
ATTATCTCTCAAAATTACAAAGTTCTCAAGGGTTTTTAGGAATAGCTAGTG  
AATTGGTAACCTATGATCAACGCTTGTCAAACATTTTT

MINKYSCFLKRILHNNTPLIVIVRSILKKNRQGRATFLPLTTIKPRELAQHYLSK  
LQSSQGFLGIASELVTYDQRLSNIF

## Sequence description

A] Length: 240 bp - 80 aa (partial sequence)

B] No obvious Shine Dalgarno sequence precedes the Putative TTG start  
codon

ID-153

## Clone 2-c94

TTGTTGACTCACAAAAATATATTATTAACCATTATATTTGGATTATTTATGA  
TTATATTATCAGCATGTGGTATGTCTAATAAGGAAATGGCTGGTATTGATA  
ATTGGGAACATTATCAAAAAGGAAAAGAAAATTACTATTGGATTGATAAT  
ACTTTTGTTCTATGGGATTTGAAAGTCGTTCTGGTGACTATACCGGCTTTG  
ATATTGATTTAGCTAATGCTGTTTTTAAAGAATACGGTATTTTCAGTGAAAT  
GGCAGCCTATTAAGTGGGATATGAAAGAACTGAACCTAATAATGGTAAT  
ATAGACCTTATTTGGAATGGTTATTCAAAAACGGCAGAACGTGCTAAAAA  
AGTCGCTTTTACAAACCCATATATGAATAATCATCAAGTAATTGTTACTAA  
AACTTCATCACATATTAATAGTATTAAGGATATGAAGGGGAAAAAACTAG  
GAGCCCAGTCGGGTTTCATCTGGTTTTTGATGCTTTTAAACGCTAAACCTGATA  
TTTTAAAAAAGTTTGTAAGGAAAAGAAAGCAGTTCAATACGATACTTTC  
ACTCAGGCTTTGATTGATTTAAAAAATAACCGTATTGATGGTCTTTTGATT  
GATGAAGTTTATGCTAACTATTATTTAAAGCAAGAAGGAA

FIG. 1<sub>CONT'D</sub>

62 / 110

MLTHKNILLTIIFGLFMIILSACGMSNKEMAGIDNWEHYQKEKKITIGFDNTFV  
PMGFESRSGDYTGFDIDLANAVFKEYGISVKWQPINWDMKETELNNGNIDLI  
WNGYSKTAERAKKVAFTNPYMNNHQVIVTKTSSHINSIKDMKGKKLGAQSG  
SSGFDAFNAKPDILKKFVKGKEAVQYDTFTQALIDLKNNRIDGLLIDEVYANY  
YLKQEG

#### Sequence description

A] Length: 649 bp - 216 aa (partial sequence)  
B] TTG start codon is preceded by a possible  
typical Shine-Dalgarno sequence. Has a  
leader peptide

ID-154

Clone 2-c100

ATGAAAATTTGGAAAAAATAACCTTAATGTTTTCTGCAATTATTTTAACA  
ACAGTAATTGCATTGGGAGTCTATGTTGCCTCAGCTTATAATTTTTCGACTA  
ATGAATTGTCTAAGACTTTT

MKIWKKITLMFSAIILTTVIALGVYVASAYNFSTNELSKTF

#### Sequence description

A] Length: 123 bp - 41 aa (partial sequence)  
B] ATG start codon is preceded by a potential  
typical Shine-Dalgarno sequence. Has a  
typical leader peptide

ID-155

Clone 2-c1

**FIG. 1** CONT'D

63 / 110

ATGAAAAAACAAGACTATTACTGCTTTTTGGAGGCTTATTAATAATGATA  
ATGATGACAGCATGTAAGGATTCAAAAATCCCAGAAAACCGCACGAAAAA  
GGAATACCAGGCAGAACAGAATTTTAAGTCATACTTTAAATATATATCAG  
ATAAAAATAACTATTTAGATAATATAAAAGTTTATTACTTTTCTATAAGTA  
TTTCTAAAGATGTACAAGATAAAGTCAGTGAAACAACAACCTTGTTTCATATA  
GACTAGAAAAGCAAAAGAATCAAGAGTTCATTGGTAATTTTGAACATGAA  
GTTAGTGAATCTAGTCAATATTCAACCGAAGTTAAAAATCAAATACAGTAT  
CCAATCCAGTATAAAGATAATTCAATTCGTTTTACTGAAAAAACACCGTCA  
GAACGTTATGATGAGTTTGTITTTAGTTCATTTGATTCTTCATTATTAATAA  
AATATAAAATATATGATTACTTACTAAAACATCCCGAAACTGAATTAATAA  
GGTGTTCCTATAAGATTCCCTATAAATTCTGAAATTGTAGCCCCTTTTATAA  
ATCAATTAAATATAAAAAAATCCTAAAAAATCATCTATTTTCGGTTACAAAAA  
CGGAAAGTAAAGAATATTATTATACAATCAGTATTGATACTGATTCTGAGA  
TATATTCTATATTCGAAGGTATTCAT

MKKQRLLLLFGGLLIMIMMTACKDSKIPENRTKKEYQAEQNFKSYFKYISDKN  
NYLDNIKVYYFSISISKDVQDKVSETTTCSYRLEKQKNQEFIGNFEHEVSESSQ  
YSTEVKNQIQYPIQYKDNSIRFTEKTPSERYDEFVFSSFDSSLLKKYKIYDYLLK  
HPETELKGVSYKIPINSEIVAPFINQLNIKNPKKSSISVTKTESKEYYYTISIDTDS  
EIYSIFEGIH

#### Sequence description

- A] Length: 687 bp - 229 aa (partial sequence)
- B] ATG start codon is preceded by a potential typical Shine-Dalgarno sequence. Has a typical leader peptide. C-terminus has yet to be verified

ID-156

Clone 2-c5

ATGACATTTGACACCATTGATCAATTAGCGGTTAATACAGTCCGCACGCTT  
TCTATTGATGCTATCCAAGCAGCAAATTCTGGGCACCCAGGTCTTCCTATG  
GGAGCTGCGCCTATGGCTTATGTGCTTTGGAATAAATTCTTAAATGTAAAC  
CCAAAAACAAGTCGCAATTGGACAAACCGTGACCGTTTTGTACTTTCAGCT

**FIG. 1** CONT'D

64 / 110

GGGCATGGTTCAGCTCTTCTTTATAGCCTACTTCATTTAGCTGGCTATGATT  
TATCAATTGATGATT

MTFDTIDQLAVNTVRTLSDAIQAANS GHPGLPMGAAPMAYVLWNKFLNVNP  
KTSRNW TNRDRFVLSAGHGSALLYSLHL LAGYDLSIDD

#### Sequence description

A] Length: 272 bp - 90 aa (partial sequence)  
B] ATG start codon is preceded by a potential  
typical Shine-Dalgarno sequence. No obvious  
leader peptide

ID-157

Clone 2-c8

ATGAGAACACTATTTAGAAATGATATTTGCTATTCCAAAGTTTATCTTTAGA  
TTGATTTGGAATATCATTG GGGGAATATTCAAGACAGTTCTTGTTATTGCG  
ATTATTTTATTTGGCTTGTATTACTATGCGAATCACAGTCAATCAGAATTG  
CTAATCAACTTAGTGACATTATTCAGACAGGAAAAACATTTT

MRTLFRMIFAIPKFIFRLIWNIIWGIFKTVLVIAIILFGLYYYANHSQSEFANQLS  
DIIQTGKTF

#### Sequence description

A] Length: 197 bp - 65 aa (partial sequence)  
B] ATG start codon is preceded by a potential  
typical Shine-Dalgarno sequence. Possesses a  
leader peptide

ID-158

**FIG. 1** CONT'D

65 / 110

Clone 2-c9

ATGTCAAAAAAATAATATTAGGAATTTTATCTCTTTTATCTGTCGTTACTT  
TGGTGGCGTGTGGTTCATCAGACAAACAGCTACAAGATAAAGTTGAGAAA  
AAAGGGAAGTTAGTTTTAGCGGTGAGTCCAGATTATGCTCCCTTTGAGTTT  
MSKKIILGILSLLSVVTLVACGSSDKQLQDKVEKKGKLVLA VSPDYAPFEF

Sequence description

A] Length: 153 bp - 51 aa (partial sequence)  
B] ATG start codon is preceded by a potential  
typical Shine-Dalgarno sequence. Possesses a  
leader peptide (not in frame with nuc)

ID-159

Clone 2-c10

ATGAAAAATCAAAGACTATTACTGCTTTTTGGAGGCTTATTAATAATGATA  
ATGATGACAGCATGTAAGGATTCAAAAATCCCAGAAAACCGCACGAAAAA  
GGAATACCAGGCAGAACAGAATTTTAAGTCATACTTT  
MKNQRLLLLFGLLIMIMMTACKDSKIPENRTKKEYQAEQNFKSYF

Sequence description

A] Length: 139 bp - 46 aa (partial sequence)  
B] ATG start codon is preceded by a potential  
typical Shine-Dalgarno sequence. Possesses a  
leader peptide

FIG. 1<sub>CONT'D</sub>

66 / 110

ID-160

Clone 2-c11

ATGATTGGAAAATTATATTATAGCTATAGAAAGTCACGCTTATTAAGAAGT  
ATTTTATGGCTTATTTTAATTGTTGGTGTATATATGTTAGGACAACGTGTTT  
TATTATCCACTGTTTCCTTTATCACATCAAGAGATAAACTAGCAGTAGATC  
AACATTTACTCAATAACTTTTCAGCAGTAAGTGGTGGGAGTTTAAATAAAT  
TAAATGTTTTTACACTGGGGTTGAGTCCATGGATGTCAAGTATGATTATTT  
GGAGATTCGTTTCCTTATTTTCGTGGGCAAAAAATGCAACGAAGCGAAAA  
GCAGAAGTAGCTCAATATACTTTAATGCTTACTATCTCAGTTATACAAGCA  
TATGGTGTTCAGGAAATCAATTTATAAAAAGCTCTTTATTAGGTTCTTATA  
GTGATATTGTTTTT

MIGKLYYSYRKSRLRSILWLILIVGVYMLGQRVLLSTVPLSHQEIKLAVDQHL  
LNNFSAVSGGSFNKLVFTLGLSPWMSSMIIWRFVSLFSWAKNATKRKAeva  
QYTLMLTISVIQAYGVSGNQFIKSSLLGSYSdIVF

Sequence description

A] Length: 423 bp - 141 aa (partial sequence)  
B] ATG start codon is preceded by a potential  
typical Shine-Dalgarno sequence. Possesses a  
leader peptide

ID-161

Clone 2-c13

ATGAAAGGTCTATTGGATTTTTTAGTTAATATTGCCAGAACGCCAGCTATT  
TTAGTCGCCTTGATAGCCATTATCGGTTTAGTACTGCAGAAAAAAGGTGTT  
CCTGATATTGTAAAAGGTGGAATAAAAAACATTTGTTGGCTTCTTAGTGGTT  
TCTGAAGGTGCAGGGATAGTCCAAAATTCCTTGAATCCATTTGGAAAAATG  
TTTGAACATGCTTTTCATTTGGTGGGGGTAGTTCCTAATAATGAAGCCATT  
GTAGCAGTAGCTCTTACGAAGTATGGCTCAGCAACTGCTTTGATTATGTTA  
CGGGAATGATTTTAAATATTTTAATTGCTCGTTTTACAAAA

FIG. 1 CONT'D

67 / 110

MKGLLDFLVNIARTPAILVALIAIIGLVLQKKGVDPDIVKGGIKTFVGFLVVSEG  
AGIVQNSLNPFGKMFEHAFHLVGVVPNNEAIVAVALTKEYGSATALIMLAGMI  
FNILIRFTK

#### Sequence description

A] Length: 348 bp - 116 aa (partial sequence)  
B] ATG start codon is preceded by a potential  
Shine-Dalgarno sequence. Possible leader  
peptide

ID-162

Clone 2-c21

TTGGTTGGTAAGCCCCAATTACTATTTTTAGATGAACCTACTTCCGGAATG  
GATACTTCCACACGTCAACGATTTTGGAAGCTGGTTGCGACACTAAAAAA  
AGAAGGTGACACAATTGTCTATTCTAGTCATTATATCGAAGAGGTAGAAC  
ATACAGCTGATAGGATTTTAGTACTTCATAAAGGAAAGTTATTACGCGATA  
CAACCCCTTTGCCATGAAGCAAGAAAAAACCGAAAAGTTATTCACCGTT  
CCGCTTAGTTATCAAAAATTATTACCTACCTATTTGATTACAGAGTGTGAA  
GCCAAGAGTGATAGTATAACGTTTGTTACTGGGGAGGCTGAAACTGTATG  
GAAAATACTGGCAGATAATGGTTGTCCTATTGAAGCTATTGAGATGACCA  
ATAGAACTTTGTAAATCGTATTTTGGAGACTACTAAGGAGGTAAAACATG  
AGAATCTTTA

MVGKPQLLFLDEPTSGMDTSTRQRFWKL VATLKKEGDTIVYSSHYIEEVEHTA  
DRILVLHKGLLRDTPFAMKQEKTEKLFTVPLSYQKLLPTYLITECEAKSDSI  
TFVTGEAETVWKILADNGCPIEAIEMTNRTLLNRIFETTKEVKHENL

#### Sequence description

A] Length: 462 bp - 155 aa (partial sequence)  
B] Putative TTG start codon is not preceded by  
an obvious Shine-Dalgarno sequence. No obvious  
leader peptide. N- and C- termini require further

**FIG. 1** CONT'D

68 / 110

examination.

ID-163

Clone 2-c25

TTGAAAAAATCCAAGAGAAGCCGTAAGGCAGTGACAACAAGTGGTGAGA  
AGACTTTACTTGAGGATTTGGCAAAAATGAATTCCTAGACGAAGTCATTA  
ATGTTATGGTTTTATATACCTTGAATAAGACAAAATCTGCTAACTTAAATA  
AGGCCTATATCATGAAAGTTGCTAATGATTTTGCCTTTCAGAATGTTATGA  
CGGCCGAAGATGCTGTGCTTAAAATTCGTGATTTTTCAGATCAAAAAGTAA  
GGACTAAAACAGAAACGAAGAAGAAACAATCGAATGTTTCCTGAATGGAGT  
AATCCTGATTATAAAGATGAGGTTAGCCCAGAAAAAGAAATTGAATTAGA  
ACAGTTT

MKKSKRSRKAVTTSGEKTLLEDLAKMNFLDEVINVMVLYTLNKTksANLNK  
AYIMKVANDFAFQNVMTAEDAVLKIRDFSDQKVRTKTETKKKQSNVPEWSN  
PDYKDEVspeKEIELEQF

Sequence description

- A] Length:360 bp - 120 aa (partial sequence)  
B] N- and C- termini require verification.

ID-164

Clone 2-c28

ATGACGAATCATATTACTAAACTGATAGAAAATAGCGGAAAAAAATTGAC  
AGAAATTAGCGAAGCTACAGATATAGCCTATCCTACACTTTCTGGATACAA  
TCAAGGAATCCGCAAACCTAAAAAAGATAATGCTGAAAAAATTGGCAAAAT  
ACTTTAATGTTTCCGTCGCTTACATTATGGGACTTGATAGCAACCCACATG  
CTCCATCAAATCTT

MTNHITKLIENSGKKLTEISEATDIAYPTLSGYNQGIRKPKKDNAEKLAKYFNV  
SVAYIMGLDSNP HAPSNL

FIG. 1<sub>CONT'D</sub>

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## Sequence description

- A] Length:218 bp - 72 aa (partial sequence)  
B] ATG start codon is preceded by an obvious Shine Dalgarno sequence. No obvious leader peptide.

ID-165

Clone 2-c29

TTGATGAAAAGGAATAAACATTTACCGTTAACAGAACTACCTATTATATT  
TTATTAGCTTTGTTTGAGGAAGCGCATGGCTATGCTATTATGAAAAAAGTT  
GAAGAAATGAGTGGCGGTGATGTTAGAATAGCCGCAGGGACAATGTACGG  
TGCCATTGAAAATTTACTTAAACAAAAATGGATAAAGTCTATCTCAAGTGA  
CGATAGAAGAAGAAAAGTTTATATTATTACTGAGACAGGAAAAGAAATAG  
TAGAACTTGAAACGAATCGATTAAGAAAGTTACTTAATACTGCTAATCAGT  
TGGGTTTTGGAGGAGATGGTTATGATAAAGTTT

MMKRNKHLPLTETTTYILLALFEEAHGYAIMKKVEEMSGGDVRIAAGTMYG  
AIENLLKQKWIKSISSDDRRRKVYIITETGKEIVELETNRLRKLLNTANQLGFG  
GDGYDKV

## Sequence description

- A] Length:337 bp - 112 aa (partial sequence)  
B] TTG start codon is preceded by an obvious Shine Dalgarno sequence. Actual start codon may ATG that comes immediately after the TTG. Potential leader peptide.

ID-166

FIG. 1 CONT'D

70 / 110

Clone 2-c35

CCCATTACTGGTGAGTTAATAGCTGAGAAATTAGGAGTACCAAGAGCAGC  
ACTAAGGTCTGATTTGCGGGTTTTAAGTATGCTAGGTATCATAGATGCAAA  
ACCTAAGGTTGGTTATTTTTATTTAGGACAGTATCATGCTTCAATAGGGAC  
AAGTCATTTTGAAAAGATGACAGTTTCAGAAATTATGGGGATCCTTCTGAC  
AGTTCATCAAAAAGATTTCAGTTTATGATGTTATTGTACATATTTTTATGGA  
AGATGCTGGTTGTGCTTTTATCTTGGATGATGATGATTTTCTCTGTGGAGTC  
GTGTCACGTAAAGATTTACTAAAAACCAGTATTGGCGGAGGAGATCTTTCT  
AAAATGCCAATAGGAATGGTGATGACACGTATGCCACACGTGACAACGTG  
TTTAGAAAATGAAAGTCTTTTTGCGGCAGCTGATAAATTAGTGAGCAGAA  
AAGTGGATAGTCTCCCTGTCGTTTCGTCATGATAAGCAATATCCCGAAAAAT  
TTA

PITGELIAEKLGVPRALRSDLRVLSMLGIIDAKPKVGYFYLGQYHASIGTSHF  
EKMTVSEIMGILLTVHQKDSVYDVIVHIFMEDAGCAFILDDDDFLCGVVSRKD  
LLKTSIGGGDLSKMPIGMVMTRMPHVTTVLENESLFAAADKLVSARKVDSLPLV  
VRHDKQYPEKF

Sequence description

- A] Length:511 bp - 170 aa (partial sequence)  
B] N- and C-termini to be determined

ID-167

Clone 2-44

TTGGAAGTCATCATGCAATTTATTTATAGTATTATTGGTATTTTATTGGTAT  
TAGGAATTGTGTATGCAATTTCTTTCAATCGTAAGAGTGTTTCTCTAAGTTT  
AATTGGAAAAGCTCTTATCGTTCAATTCATTATTGCGCTAATCTTAGTACGT  
ATCCCACTAGGCCAACAAGTTGTTAGTGTTGTTCAACTGGAGTTACTAAA  
GTAATCAACTGTGGTCAAGCTGGTTT

MEVIMQFIYSIIGILLVLGIVYAISFNRKSVSLSLIGKALIVQFIHALILVRIPLGQQ  
VVSVVSTGVTKVINCGQAG

**FIG. 1** CONT'D

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## Sequence description

- A] Length:233 bp - 77 aa (partial sequence)  
B] TTG start codon is preceded by a possible Shine Dalgarno sequence. Actual start codon may occur further downstream. Potential leader peptide.

ID-168

Clone 2-46

CAACCTAATAAAGCTTTAGAAAGTGATGAGATTGATATTAATGCTTTCCAG  
CATTATAATTACTTAACCAATTGGAATAAAGCAAATAAGACCAATCTTGTT  
TCCGTTGCTGAGACATACTTTACTTCCTTTAGATTATACTCTGGTACTAAGA  
ACGGTAAAGGTAAATACCAAACAGTTTCTGAAATTCCAAATAAAGCAACT  
ATTACTATCCCAAACGATGCAGTTAACGAAAGTCGCTCTCTCTACTTGTTA  
CAATCAGCAGGCTTGCTAAAATTGAAAGTATCAGGTGATACATTAGCAAC  
AATGTCAGATGTTGTTTCCAATCCTAAATCTTTAGATT

QPNKALESDEIDINAFQHYNLTNWNKANKTNLVSVAETYFTSFRLYSGTKN  
GKGKYQTVSEIPNKATITIPNDVNESRSLYLLQSAGLLKLKVSGDTLATMSD  
VVSNPKSLD

## Sequence description

- A] Length:344 bp - 114 aa (partial sequence)  
B] N- and C- termini require verification

ID-169

Clone 2-47

ATGAAATGTATAATAATAATATAAATAAAATAAAAAATGATAATTGAGAT  
TTATCATAGAAGGAAAACATTTTGAATTAATAAAATCATATTATCTAC

FIG. 1<sub>CONT'D</sub>

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TGCAGCTCTTACTGCTCTCTTTTTAGGATATAATAGCGTTACTGCGGATACA  
TATAAATACTATCAGCCACATAGATCAAATAATATGGATTTAAGTGAAGGA  
ATATAACTATAATAACCAGATAGAACTTCAGGAGCGTATAAAAAACCTAA  
ATATACCTTTT

MKCIINNINKIKMIIIEIYHRRKTILKLNKIILSTAALTALFLGYNSVTADTYNNY  
QPHRSNNMDLTEEYNNNQIELQERIKNLNIPF

#### Sequence description

- A) Length:264 bp - 88 aa (partial sequence)
- B) There is a Shine-Dalgarno sequence upstream of this sequence. Potential leader peptide sequence

ID-169

Clone 2-47

ATGAAATGTATAATAAATAATATAAATAAAAAATGATAATTGAGAT  
TTATCATAGAAGGAAAACCTATTTTGAAATTAATAAAAAATCATATTATCTAC  
TGCAGCTCTTACTGCTCTCTTTTTAGGATATAATAGCGTTACTGCGGATACA  
TATAAATACTATCAGCCACATAGATCAAATAATATGGATTTAAGTGAAGGA  
ATATAACTATAATAACCAGATAGAACTTCAGGAGCGTATAAAAAACCTAA  
ATATACCTTTT

MKCIINNINKIKMIIIEIYHRRKTILKLNKIILSTAALTALFLGYNSVTADTYNNY  
QPHRSNNMDLTEEYNNNQIELQERIKNLNIPF

#### Sequence description

- A) Length:264 bp - 88 aa (partial sequence)
- B) There is a Shine-Dalgarno sequence upstream of this sequence. Potential leader peptide sequence

**FIG. 1** CONT'D

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ID-170

Clone RS-58b

TTGGGTGATTATTATGGTAAGAAATATTTTGGTGAGGCAGCTAAAAAAGA  
 CGTCGAACATATGGCTAAGAAAATCATTAATGTCTATAAAACACGGTTAA  
 AAAACAACACTTGGTTATC  
 AGAAAATACAAAAGCAATGGCCATTAAGAACTTGATAACATGAGATTAA  
 TGATTGGCTATCCAGAAGATTATCCTGATCTTTATCGTCAGTACCAATTTG  
 ATAGTAAAGCAAGCTTCTTTGAAAACAATGATAACTACAGAAAATTATCG  
 AACAAGAAAACATTTGAAGAATTTAACCAGTCTAATCAACGTGAACATTG  
 GCAAATGAGTGCCAATGCTGTAAATGCTTATAATGATCCTAATACCAATTC  
 CATAGTCTTTCCAGCAGCGATTTTCAATCACCAGTGTACGATAAACTAA  
 AACAGTTAGTCAAAATTATGGAGCTATCGGAGCAATTATTGGTTCATGAAAT  
 TTCACACTCATTGATATTAATGGTATGAAATATGACGAGAAAGGGAATCT  
 TCACGATTGGTGGACTAAAGAAGATTTAAATCATTATAAGAAATCAACAC  
 AAGCTATGATTGACCAATGGGATGGCCTTAAAGCAGATGGCGGTAAAGTT  
 GATGGTAAATTAACCTTAGCAGAAAATATTGCAGATAATGGTGGTGTATG  
 GCATCTCTAGAAGCTCTTAAGACTGAAAAAATCCAACTATAAAGAATTTT  
 TTGAATCATGGGCAAGTATTTGGCGTCAAAAAGCAACCAAAGAACAAGT  
 AAGTCCTCAATTCAGTCAGATGTTTCATGCACCATATGAATTGA >  
 GAGCTAACATCCCAGTACGTAATTTCCAAGAATTTTATGATGCCTTTGGTG  
 TTA AAAAAGGCGATTCAATGTATCTAAAACCAGAAAAACGTTTGACACTTT  
 GGTA

MGDYYGKKYFGEAAKKDVEHMAKKIINVYKTRLKNNTWLSENTKAMAIAKK  
 LDNMRLMIGYPDYPDLRQYQFDSKASFFENNDNYRKLSNKKTFEEFNQSNQ  
 REHWQMSANAVNA YNDPNTNSIVFPAAIFQSPLYDKTKTVSQNYGAIGAIIH  
 EISHSFDINGMKYDEKGNLHDWWTKEDLNHYKKSTQAMIDQWDGLKADGG  
 KVDGKLTLENIADNGGVMASLEALKTEKIQTIKNFLNHGQVFGVKKQPKNK  
 VSPQFSQMFMHHMN\*

Sequence description:

A] Length: 819 bp - 272 aa (full length gene)  
 (107 bp of additional DNA sequence (> onwards) is  
 also included. While not in-frame with the  
 described orf, it also shares strong homology  
 with the neutral peptidases.

FIG. 1<sub>CONT'D</sub>

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B] This gene sequence was not identified using the LEEP system. It was identified downstream of the ID-89 gene which was identified by LEEP, during cloning and sequence analysis of the full-length ID-89 gene sequence. ID-89 and ID-170 together show homology over their combined entire length with the neutral endopeptidases from *Lactococcus* and *Lactobacillus*. Possesses TTG (possible ATG start codon located 13 bp further downstream) start codon with no obvious signal peptide. Shine Dalgarno sequence not immediately obvious. Possibly located further downstream

ID-171

Clone 2-18/22b (Mod2)

ATGACCATGATTACGCCAAGCTTCATTAAGGTATCTCTAGATGAAACAAAT  
 CGTATGATGCGTATGATATCAGATTTATTAAGTTTATCGCGCATTGATAAT  
 GAAGTAACGCATTTAGATGTTGAAATGACGAATTTTACAGCTTTCATGACC  
 TCAATTTTGAATCGATTTGATCAGATTAGAAATCAAAAAACAGTCACAGG  
 AAAAGTTTATGAAATTGTCAGAGATTATCCTCTTAAGTCAATTTGGGTGGA  
 AATTGATACAGATAAGATGACTCAAGTGATTGATAACATTTTAAATAATGC  
 AGTCAAGTATTCACCAGATGGTGGTAAGATTACAGTTAATCTACGCACAAC  
 TAAAACGCAGATGATTTTATCAATATCAGACCAAGGCTTAGGTATTCCCAA  
 AAAAGATTTACCTCTCATTTTTGATCGTTTTTATCGTGTTGATAAGGCGAGA  
 AGTCGTCAACAGGGTGGGACTGGACTTGGTTTGTCAATTGCAAAAGAAAT  
 TGTTAAGCAGCATAAGGGATTTATTTGGGCTAAGAGTGAGTATGGTAAAG  
 GGTCTACTTTTACAATCGTCTTGCCTTATGATAAAGATGCTGTAACCTTATGA  
 AGAATGGGAGGACGTTGAAGATTAA

MTMITPSFIKVSLDETNRMMRMISDLLSLSRIDNEVTHLDVEMTNFTAFMTSIL  
 NRFDQIRNQKTVTGKVYEIVRDYPLKSIWVEIDTKMTQVIDNILNNAVKYSP  
 DGGKITVNLRTTKTQMILSISDQGLGIPKKDLPLIFDRFYRVDKARSRQQGGTG  
 LGLSIAKEIVKQHKGFIWAKSEYKGKSTFTIVLPYDKDAVTYEEWEDVED\*

Sequence description:

- A] Length: 613 bp - 212 aa (full-length gene possibly)
- B] Possible Shine Dalgarno sequence present upstream of a ATG start codon. May not have yet determined the N- portion of this gene. No obvious signal peptide.

FIG. 1<sup>CONT'D</sup>

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ID-172

Clone 2-54balternate (107b)

TTGAAAAAAATTATTACTTCTATTCTATTACTTAGTTGCATTTTTTTTATGC  
CAACCATCTCTGCTGAATCTTTTAATGCTTCCGCTAAACATGCCTTAGCAGT  
TGATTTAGATTCAGGAAAAATCTTGTATGAAAAAGATGCTAACAAACCCG  
CTGCTATTGCTTCCTTGACTAAAATAATGACCGTTTATATGGTCTATAAAG  
AAATTGATAACGGTAACCTCAAGTGGAATACCAAAGTAAATATATCTGAC  
TACCCTTATCAACTAACACGCGAATCTGATGCTAGTAATGTTCTTTAGAA  
AAAAGGCGCTATACTGTAAACAACCTCGTGGACGCTGCCATGATTTCTAGT  
GCTAACAGTGCAGCCATTGCTTTAGCTGAACATATTTTCAGGAAGTGAAGT  
AAATTTGTTGATAAAATGACTGCTCAATTGGAAAAGTGGGGAATTCATGAT  
AGCCACCTAGTCAATGCTTCTGGCTTAAATAATAGTATGTTAGGCAATCAC  
ATTTATCCAAAATCGTCACAAAACGACGAAAATAAAATGAGTGCACGTGA  
TATTGCTATTGCTGCCTACCATTTGGTCAACGAATATCCTTCCATTCTTAAG  
ATTACTAGTAAGTCCGTTGCTAAATTTGATAAAGATATTATGCATTCTTAT  
AACTACATGCTACCAGATATGCCTGTCTTTAGACCAGGTATTACAGGTTTG  
AAAAGTGGGACAACGGAATTAGCTGGCCAATCTTTTATTGCTACATCTACT  
GAAAGTGGAATGAGACTACTCACTGTTATTATGCATGCTGATAAGGCCGAT  
AAAGACAAATATGCTCGCTTTACAGCAACTAACTCTCTCTTGAAGTATATC  
ACAAACACCTACGAACCTAACCTTGTATTAGCTAAAGGAGCTGCATATAA  
AGGTAAAGAAGCAAGTGTGAGAGACGGAAAAGAACAATCGGTCATCGCT  
GTTGCTAAAAACGATTTGAAAGTAGTACAGAAGAAAAAATATCACTAAACA  
AAATCAGTTAAAAATTAACCTTTAAAAAAGAGCTTACTGCTCCTATTACAAA  
AAAAGAGAACCTAGGGAAAGCTTATTACGTTGACCTTAATAAGGTTGGAA  
AAGGCTATCTCATAAAGGAACCTAGCGTTCATTTAGTGGCAAAGATAGT  
ATTGAGCGCAGTTTCTTCCTCAAAGTGTGGTGGAATCATTTTGTGCGCTAC  
GTTAACGAAAAACTTTAA

MKKIITSILLLSCIFFMPTISAESFNASAKHALAVDLDSGKILYEKDANKPAAIA  
SLTKIMTVYVMVYKEIDNGNLKWNTKVNISDYPYQLTRESNASNVPLEKRRYT  
VKQLVDAAMISSANSAAIALAEHISGTESKFVDKMTAQLEKWGIHDSHLVNA  
SGLNNSMLGNHIYPKSSQNDENKMSARDIAIAAYHLVNEYPSILKITSKSVAKF  
DKDIMHSYNYMLPDMVFRPGITGLKTGTTELQSFATSTESGMRLTLVIM  
HADKADKDKYARFTATNSLLNYITNTYEPNLVLAKGAAYKGKEASVRDGKE  
QSVIAVAKNDLKVVQKKNITKQNQLKINFKKELTAPITKKENLGKAYYVDLN  
KVGKGYLIKEPSVHLVAKDSIERSFFLKVWWNHFVRYVNEKL\*

FIG. 1 CONT'D

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## Sequence description:

- A] Length: 1236 bp - 412 aa (full-length gene sequence possibly)  
 B] A possible Shine-Dalgarno sequence precedes the putative 'TTG' start codon. (needs further cloning and sequencing to verify N-terminus)

ID-173

Clone 3-60b

ATGACGCTTCGAGAATTAACAATAGAAGAATTTAAAGAACATTCAGGAAA  
 TTATGATTCACAATCATTTTTACAAACACCTGAGATGGCTAAACTTTTAGA  
 AAAACGCGGCTATGATGTTAGGTATTTGGGATATCAAGTAGAAAATAAAC  
 TAGAGATAATCAGTTTATCTTATATTATGCCAGTCACTGGTGGTTTTCAAAT  
 GAAAATTGATTCAGGACCAGTTCATTCAAATTCTAAGTATCTAAAACAATT  
 TTATAAAGCATTGCAAGGCTATGCCAAATCCAACGGTGTCTAGAAATTAAT  
 AGTTGAGCCTTTTGATGATTACCAATTATTCAGTTCGGGAGTTCCTAGT  
 AATCAGGGAAATGATAATCTGATTGAAGATTTTACCAGTTCAGGTTATCAC  
 CATGATGGTTTAACAACCTGGTTTTACTGGTAAATATTTATCTTGGCACTATG  
 TTAAAAATTTAGAAGGTGTCACCTTCTGAAACGTTACTATCTTCATTCTCTAA  
 GACAGGACGAGCTTTGGTTAAGAAAGCAATGTCTTTTGGAATCAAGGTTC  
 GCGTTCCTTAAACGTGATGAGCTACATTTATTTAAAGAGATAACAACCTTCTA  
 CGTCAAATAGACGTGATTATATGGATAAGTCCTTAGATTATTATCAAGATT  
 TTTACGATAGCTTTGAAGGCAAGGCTGAATTTGTGATTGCCACTTTAAATT  
 TTAGAGAATACGACCATAACTTGCAAATAAAAGCTGAAGCATTGGAAAAT  
 AAGCTT

MTLRELTIEEFKEHSGNYDSQSFLQTPEMAKLLEKRGYDVRYLGYQVENKLEI  
 ISLSYIMPVTGGFQMKIDSGPVHSNSKYLKQFYKALQGYAKSNGVLELIVEPF  
 DDYQLFTSSGVP SNQGNLIEDFTSSGYHHDGLTTGFTGKYLSWHYVKNLE  
 GVTSETLLSSFSKTGRALVKKAMSFGIKVRVLKRDELHLFKEITTSTSNRRDY  
 MDKSLDYYQDFYDSFEGKAEFVIATLNFREYDHNLQIKAEALENKL

## Sequence description

- A) Length: 771 bp - 257 aa (partial gene sequence)  
 B) This gene sequence was not identified using the LEEP system. It was identified immediately downstream of the ID-65 gene which was identified by

FIG. 1 CONT'D

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LEEP, during cloning and sequence analysis of the full-length ID-65 gene sequence. Sequence Characteristics:  
 No obvious leader peptide sequence  
 Orf is preceded by a potential Shine-Dalgarno sequence.

ID-174

Clone 2-17b (ID-80b)

TTGTCATTAAGTTTGGTTGCAGTGTTAAATCTTATCCCTCCTAAAATCATGG  
 GATCAGTTATTGATGCTATTACAACCTGGAAAATTAACAAGACCACAATTAC  
 TATGGAATTTATTAGGTTTGGTTTTGTCAGCTTTAGCTATGTATGGGCTGCG  
 TTATATTTGGCGTATGTATATTTAGGGACTTCTTACAAATTAGGCCAAGTT  
 GTCAGATACCGTTTATTTGAACATTTTACAAAAATGTCTCCTTCTTTTTATC  
 AGAAATATCGTACAGGTGATTTAATGGCGCACGCGACCAACGACATCAAT  
 TCTCTAACACGTCTTGCAGGAGGAGGAGTTATGTCAGCAGTGGATGCCTCT  
 ATCACAGCATTAGTAACGCTTATCACCATGTTCTTTACTATTTTCGTGGCAA  
 ATGACATTAATTGCGGTTATCCCTTTGCCCTTAATGGCCTTAGCACTAGTA  
 AATTGGGGCGAAAAACCCATGAAACCTTCAAAGAATCTCAGGCAGCCCTT  
 TTCAGAATTAATAATAAAGTG

MSLSLVAVLNLIPPKIMGSVIDAITTGKLTRPQLLWNLLGLVLSALAMYGLRYI  
 WRMYILGTSYKLGQVVRYRLFHFTHKMSPSFYQKYRTGDLMAHATNDINSLT  
 RLAGGGVMSAVDASITALVTLITMFFTISWQMTLIAVIPLPLMALALVNWGEK  
 PMKPSKNLRQPFSELNNKV

#### Sequence description

A) Length: 534 bp - 178 aa (partial gene sequence)

B) This gene sequence was not identified using the LEEP system. It was identified downstream of the ID-80 gene which was identified by LEEP, during cloning and sequence analysis of the full-length ID-80 gene sequence.

Sequence Characteristics:

No obvious leader peptide sequence

Orf is preceded by a potential Shine-Dalgarno sequence.

## FIG. 1<sub>CONT'D</sub>

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ID-175

Clone 2-11Ab (ID-103b)

ATGCATATTGAGACTGTTATTGATTTCAAAGAATTAGGAAAAAGATATCGT  
TTTAAAAATCCTACAAAAGAATTAATAGCTGATACTTTAGAACAAGTCTTA  
GAAGTGATAAAAGAAGTTGATTATTATCAATCTCAAATTATTATGTTGTT  
GGTTATTTATCTTATGAAGCATCTGCTGCTTTTGATTCACATTTTAAAGTTT  
CTCAACAGAAGTTGGCTGGGAGAACATCTAGCTTATTTTACAGTACATAAAG  
ATTGTGAGAACGAAGCTTTTCCTTTAAGTTATGAAAATGTTAGATTAGCAG  
ATAATTGGACTGCTAATGTTTCTGAGCAAGAATATCAAGAGGCAATTGCTA  
ATATTAAAGGACAAATTAGACAAGGAAATACTTATCAAGTAAATTATACA  
CTAGAGCTTAGCCAACAATTATGCTCGGATCC

MHIETVIDFKELGKRYRFRKNPTKELIADTLEQVLEVIKEVDYYQSQNYVVG  
LSYEASAAFD SHFKVSQQLAGEHLAYFTVHKDCENEAFPLSYENVRLADNW  
TANVSEQEYQEAIANIKGQIRQGNTYQVNYTLELSQQLCSD

Sequence description:

A] Length: 440 bp - 146 aa (partial gene sequence)

B] This gene sequence was not identified using the LEEP system. It was identified downstream of the ID-103 gene which was identified by LEEP, during cloning and sequence analysis of the full-length ID-103 gene sequence. Shine Dalgarno sequence present upstream of ATG start codon, No apparent leader peptide sequence

ID-176

Clone 2-18/22b(b) (ID-104b)

GTGAATAATATGTTTTATCTCAAATAGCCTGGCATAATTTAAAACATTCT  
ATAGACCAGTACATAACCTTCTTAGCCAGTTTATTACTTTATTCATTGA  
CTTGTTCTACGCTACTAATCTTAATGAGTGCTGTTGGAAGAGATATGGGGA  
CAGCGGCAACGGTTCTTTTCTTGAGTGATTGTTTTGTCAATCTTTGCGGT  
AGTCATGGAACATTATAGCTACAATATCTTGATGAAACAGCGTAGTAGTG

FIG. 1<sub>CONT'D</sub>

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AATTTGGACTGTATAACATTTTGGGGATGAATAAACGTCAAGTTGCGCGTG  
 TAGCTAGTCTAGAGCTGTTTATTATTTATATATTTCTTATTTCTATAGGAAG  
 TCTGTTTAGTGCTTTTTTTGCTAAATTTATTTATTTAATTTTTGTCAACATTA  
 TTAACATATCATGCACTAAATCTTAGTTTAAGTTTATGGCCATTTATTATTTG  
 TATCGTTATATTTACAGGTATTTTCTGACTTTAGAAGTTCCAGTTATTCGA  
 CATGTTCAATTTATCATCCCCATTAAGTCTTTTGTAGAAAGAAACAACAGGGA  
 GAAAAAGAACCAAAAGGTAATCTTATACTTGCAATTTTAGCGTTAGTAGCT  
 ATCGCCATCGCTTATACAATGGCTCTTACTTCAGGTAAAGCACCTGCATTA  
 GCTGTTATCTATCGTTTCTTCTTTGCAGTACTTTTAGTAATTGCTGGTACTT  
 ATCTTTTTTATATTAGTTTTATGACATGGTACTTAAAAAGGTTGCGTCAAAA  
 CAAGCATTATTATTATAAATCTGAGCATTTTGTATCAACTTCGCAAATGAT  
 TTTTCGAATGAAGCAAAATGCAGTAGGGTTAGCAAGTATCACTTTATTAGC  
 TGTTATGGCTCTAGTTACTATTGCTACAACAGTCTCACTCTATTCAAATACA  
 CAAAATGTTGTTACCGGACTATTTCCAAAATCAGTAAGTTTATCAATAGAT  
 AATTCAAAGGTGACGCGAAAAATATATTTGAAGAAAAGATTTTGAAGAA  
 ACTAGGTAAGTCATCTAAGGAAGCTATCACTTATAATCAGACAATGATTC  
 GATGCCAGTTAGTCAATCAAGTGACTTAATATCACATCTA

MNNMFYFKIAWHNLKHSIDQYIPFLASLLLYSLTCSTLLILMSAVGRDMGTA  
 ATVLFLGVIVLSIFAVVMEHYSYNILMKQRSSEFGLYNILGMNKRQVARVASL  
 ELFIYIFLISIGSLFSAFFAKFIYLFVNINYNHALNLSLSLWPFIIIVIFTGIFLTLE  
 VPVIRHVHLSSPLSLFRKKQQGEKEPKGNLILAILALVAIAIAYTMALTSGBKAP  
 ALAVIYRFFFAVLLVIAGTYLFYISFMTWYLRKRLRQNKHYYYKSEHFVSTSQM  
 IFRMKQNAVGLASITLLAVMALVTIATTVSLYSNTQNVVTGLFPKSVSLSIDNS  
 KGDAKNIFEEKILKKLGKSSKEAITYNQTMISMPVSQSSDLISHL

#### Sequence description:

- A] Length: 1119 bp - 373 aa (partial gene sequence)
- B] This gene sequence was not identified using the LEEP system. It was identified upstream of the ID-104 gene which was identified by LEEP, during cloning and sequence analysis of the full-length ID-104 gene sequence. Possible Shine Dalgarno sequence present upstream of a GTG start codon. Possesses a potential leader peptide sequence

ID-177

FIG. 1<sup>CONT'D</sup>

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Clone 2-5b (ID-112b)

ATGGTTGAGCCAATTATTTCAATACAAGGACTTCATAAAAAGTTTTGGGAAA  
 AATGAGGTTTTAAAAGGCATTGACTTGGATATTCATCAAGGAGAAGTGGT  
 GGTTATTATTGGCCCTTCTGGCTCTGGTAAGTCAACATTTTAAAGAACAAT  
 GAATCTCTTGGAAGTACCAACAAAGGGAACAGTGACTTTTGAAGGGATTG  
 ATATAACAGACAAAAAGAATGATATTTTAAAATGCGCGAAAAAATGGGC  
 ATGGTTTTTCAACAGTTCAATCTATTTCCCAATATGACTGTACTAGAAAAT  
 ATTACTTTATCACCTATTAAGACAAAGGGACTTTCTAAGCTTGATGCTCAG  
 ACAAAGCATACGAGCTACTTGAAAAAGTTGGACTCAAAGAGAAGGCTAA  
 TGCTTATCCAGCAAGCTTATCTGGAGGACAACAACACGGATTGCTATTGC  
 AAGAGGTCTTGCAATGAATCCTGATGTCCTTCTTTTGGATGAACCTACTTCA  
 GCTCTTGATCCTGAAATGGTAGGTGAAGTCTTGACTGTTATGCAAGATTTA  
 GCTAAATCTGGTATGACGATGGTTATTGTCACCTCATGAAATGGGTTTTGCA  
 CGTGAAGTAGCGGATCGTGTCAATTTATGGATGCAGGGATTATTGTTGAG  
 CAAGGGACCCCTAAGAAAGTATTTGAGCAGACAAAAGAAATCCGCACAAG  
 AGACTTCTTAAGTAAAGTATTATAA

MVEPIISIQGLHKSFGKNEVLKGIDLDIHQGEVVVIIGPSGSGKSTFLRTMNLLE  
 VPTKGTVTFEGIDITDKKNDIFKMREKMGMMVFQQFNLFNMTVLENITLSPIKT  
 KGLSKLDAQTKAYELLEKVGLKEKANAYPASLSGGQQQRIAIARGLAMNPDV  
 LLFDEPTSALDPEMVGEVLTVMQDLAKSGMTMVIVTHEMGFAREVADRVIF  
 MDAGIIVEQGT PKKVFEQTKEIRTRDFLSKVL\*

Sequence description:

- A] Length: 735 bp - 244 aa (full length gene)
- B] This gene sequence was not identified using the LEEP system. It was identified downstream of the ID-112 gene which was identified by LEEP, during cloning and sequence analysis of the full-length ID-112 gene sequence. Shine-Dalgarno sequence precedes the 'ATG' start codon. No obvious leader peptide

ID-178

Clone 2-5c (ID-112c)

FIG. 1<sub>CONT'D</sub>

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ATGTCTCA<sup>s</sup>TATCAAGAGTGGTTAGAAAACGACTCACTCGGTAAAGATATT  
 AAGTCAGATTTAGAAGCTATTAAAGGAGATGAATCTGAAATTCAGGATCG  
 TTTTACAAAACATTAGAATTTGGAACGGCGGGATTGAGAGGTAAACTTG  
 GAGCAGGAACCAATCGTATGAATACTTATATGGTGGGGAAAGCAGCACAA  
 GCATTAGCTAATCGATTATTGATCATGGCCCTGAAGCTATTGCACGTGGAA  
 TTGCAGTTAGTTATGATGTCCCGTTATCAATCTAAGGAATTTGCAGAATTA  
 ACTTGGTCCATTATGGCAGCAAATGGTATTAAAGCCTTATATTTA

MSHMNYKEIYQEWLEND<sup>s</sup>SLGKDIKSDLEAIKGDESEIQDRFYKTLEFGTAGLR  
 GKL<sup>s</sup>GAGTNRMNTY<sup>s</sup>MVGKAAQALANRLLIMALKLLHVELQLVMMSRYQSKE  
 FAELTWSIMAANGIKALYL

#### Sequence description:

A] Length: 366 bp - 122 aa (partial gene sequence)

B] This gene sequence was not identified using the LEEP system. It was identified downstream of the ID-112 gene which was identified by LEEP, during cloning and sequence analysis of the full-length ID-112 gene sequence. Shine-Dalgarno sequence preceded the 'ATG' start codon. No obvious potential leader peptide sequence.

ID-179

Clone 2-5d (ID-112d)

ATGCAACCTGTAAAAGTCGATGAACCTTCTGTTGAAGAAACCATTACTATT  
 TTGAAAGGTATCCAAAAAATACGAAGATTATCATCACGTAAAATATAA  
 TAATGATGCCATAGAAGCAGCTGCAGTACTATCTAATCGTTATATCCAAGA  
 CCGCTTTTACCTGATAAAGCAATAGACTTATTAGATGAAGCTGGTTCTAA  
 AATGAACCTAACAATAATTTTGTGATCCAAAAGAAATTGATCAACGTCT  
 CATTGAAGCAGAAAATTTAAAAGCGCAAGCGACTCGTGAAGAAGATTACG  
 AACGTGCAGCTTACTTCCGTGACCAGATTGCAAAATATAAAGAAATGCAG  
 CAACAAAAGGTGACGATCAAGATACACCTATTATTACCGAAAAACAAT  
 TGAGCACATCATTGAAGAAAAAACGAATATCCCTGTTGGTGATTTAAAAG  
 AAAAAGAACAATCTCAATTAATTAATCTCGCAGATGACTTGAAACAGCAT  
 GTGATCGGCCAGGATGACGCTGTCATTAAGATTGCAAAAGCTATTCGTCTG  
 AATCGAGTTGGTCTTGGTAGCCCAAACCGTCCTATTGGTTCCTTTTATTG  
 TAGGACCAACCGGTGTTGGTAAAACTGAACCTTCTAAACAACCTAGCAATTG  
 AGCTCTTTGGTTCAGCTGATAGTATGATTCGTTTTGATATGTCAGAGTACAT  
 GGAAAAGCATGCTGTTGCTAAATTAGTCGGAGCGCCTCCAGGATACGTGG  
 GATACGAGGAAGCTGGACAACTAACTGAAAAGGTTTCGTGAAATCCTTAC  
 TCGCTCATCCTTCTAGATGAAATTGAAAAAGCTCATCCCGATGTCATGCAT

FIG. 1<sup>CONT'D</sup>

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ATGTTCTTGCAGGTCCTTGATGACGGTCGATTAACAGATGGACAAGGAAG  
 AACTGTTAGTTTTAAAGATAACCATTATCATCATGACCTCAAATGCTGGTTC  
 TGGTAAACTGAAGCAAGTGTTGGCTTTGGTGCCTCACGAGAAGGTAGGA  
 CGAATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCAT  
 GCAAGC

MQPVKVDEPSVEETITILKGIQKKYEDYHHVKYNNDAIEAAAVLSNRYIQDRF  
 LPDKAIDLLDEAGSKMNLTLNFVDPKEIDQRLIEAENLKAQATREEDYERAAAY  
 FRDQIAKYKEMQQQKVDDQDTPITEKTIEHIIIEKTNIPVGDLEKEQSQLNL  
 ADDLKQHVIGQDDAVIKIAKAIRNRNVGLGSPNRPIGSFLFVGPTGVGKTELSK  
 QLAIELFGSADSMIRFDMSEYMEKHAVAKLVGAPPGYVGYEEAGQLTEKVRR  
 NPYSLILLDEIEKAHPDVMHMFLLQVLDDGRLTDGQGRTVSFKDTIIMTSNAGS  
 GKTEASVGFASREGRTNSSSVPGDPLESTCRHAS

#### Sequence description:

A] Length: 1070 bp ÷ 356 aa (Partial gene sequence)

B] This gene sequence was not identified using the LEEP system. It was identified upstream of the ID-112 gene which was identified by LEEP, during cloning and sequence analysis of the full-length ID-112 gene sequence. Shine-Dalgarno sequence preceded the 'ATG' start codon. No obvious potential leader peptide sequence.

ID-180

Clone 2-7b (ID-113b)

ATGAGAGGGAAGGTTATTTACGGCACAACCCTTATAGGTCTTTTTCTATTC  
 TTATTTTTCTATTTTTGGATTCCCTAAGCATCACATCGAGAGAATACATCATC  
 ATCGTATAAAGCAGGTAGATGCGAAGAGTGATTTAACAGGATTTAAAACC  
 CATTTGCCCATTCATCAGCATTGATACAAAGCAACAAGTTATTCCTCTTGTT  
 ACAAAGAAGGCGGAAAATATGTCAAAGCTAGGGATAATATTAATGTTGA  
 TATCGAATTACGGGATTCTCCAAGTAGATCACATCATTTATCAGAAAAGCC  
 GAGAATTAGGACAAAAGGGTTAATATCATATAGAGGAAATTCCTCTCGTT  
 ACTTTGATAAGAAGTCATTGAAAGTTAAGTTTGTTACTAATAAGTTAAAGG  
 AAAAGAAGCATCGATTAGCAGGAATGCCTAAAGAATCGGAGTGGGTATTG  
 CATGGTCCCTTTCTAGACAGAACATTATTAAGAAATTATCTGAGTTATAAT

**FIG. 1** CONT'D

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ATTGCTGGTGAGATTATGCCTATGCCCCAAACGTTTCGCTACTGTGAGTTAT  
TTGTCAATGGTGAGTATCAGGGAG

MRGKVIYGTTLIGLFLFLFFYFWIPKHHIERIHHHRIKQVDAKSDLTGFKTHLP  
SIDTKQQVIPLVTKEGGKYVKARDNNVNDIELRDSPSRSHHLSEKPRIRTKGLIS  
YRGNSSRYFDKSLKVKFVTNKLKEKKHRLAGMPKESEWVLHGPFLDRTLLR  
NYLSYNIAGEIMPMPQTFATVSYLSMVSIRES

#### Sequence description:

- A] Length: 582 bp - 194 aa (Partial gene sequence)
- B] This gene sequence was not identified using the LEEP system. It was identified downstream of the ID-113 gene which was identified by LEEP, during cloning and sequence analysis of the full-length ID-113 gene sequence. ATG start codon is preceded by a Shine-Dalgarno sequence-Possesses a potential leader peptide sequence. C-terminus to be determined.

ID-181

Clone 2-17b (ID-117b)

CTTCACATTTTATTGATCACTATCTGACAAATGTTAATCAAACAGCAGTTCT  
TATTTTAGTGGGATATTATTCAATGTATGTCTTGCAGACCTTAATTCAATAT  
TTTGGGAATCTCTTTTTTGC GCGTGTCTTATAGTATTGTTAGAGATATTC  
GTAGAGATGCTTTTGCTAATATGGAAAGGCTAGGCATGTCTTATTTTGATA  
GGACACCGGCAGGATCTATTGTGTCACGTATTACTAATGATACTGAAGCAA  
TATCTGATATGTTTTCGGGTATTTTATCAAGTTTTATCTCGGCGATATTTAT  
TTTTACAGTTACTCTGTACACTATGTTGATGCTAGACATTAACTAACAGG  
ACTCGTCGCTCTTTTGTTACCTGTTATCTTTATATTAGTGAATGTCTATCGG  
AAAAAATCAGTCACTGTCATTGCTAAAACGAGAAAGTTTACTTAGTGATATC  
AACAGTAAATTATCAGAAAGTATTGAAGGAATTC

SHFIDHYLTNVNQTA VLILVGYYS MYVLQTLIQYFGNLFFARVSYSIVRDIRRD  
AFANMERLGMSYFDRTPAGSIVSRITNDTEAISDMFSGILSSFISAIFITVTLYT  
MLMLDIKLTGLVALLLPVIFILNVYRKKS VTVIKTRSLSDINSKLSESI EGI

## FIG. 1 CONT'D

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## Sequence description:

A] Length: 498 bp - 165 aa (Partial gene sequence)

B] This gene sequence was not identified using the LEEP system. It was identified downstream of the ID-117 gene which was identified by LEEP, during cloning and sequence analysis of the full-length ID-117 gene sequence. N- and C-termini have yet to be determined

ID-182

Clone 3-8b (ID-120b)

ATGTACCATATTGAATTAAAAAAGGAAGCTTTACTACCAAGAGAACGCCT  
 AGTTGATTTAGGCGCAGATAGATTGAGTAATCAGGAGTTATTAGCCATTCT  
 CTTACGTACAGGTATTAAAGAAAAACCTGTTCTTGAAATTTCAACGCAAAT  
 TTTAGAAAACATAAGCAGTTTAGCAGATTTTGGTCAATTATCCTTACAGGA  
 GTTGCAATCCATTAAAGGAATCGGTCAGGTTAAATCCGTCGAAATAAAAG  
 CTATGCTAGAACTAGCAAAACGGATTCACAAAGCTGAATATGATCGTAAA  
 GAGCAAATTTTAAGTAGTGAACAATTAGCGAGGAAAATGATGCTCGAATT  
 AGGGGATAAAAAACAAGAACATTTAGTAGCTATTTATATGGATACACAAA  
 ATCGTATTATCGAACAGAGAACTATTTTTATTGGTACTGTACGTCGTTTCAG  
 TAGCAGAGCCAAGAGAAATTCTACATTATGCTTGTA AAAACATGGCAACT  
 TCTTTGATTATTATACATAATCATCCCTCAGGTTCTCCAAATCCCAGTGAAA  
 GTGATTTAAGTTTCACTAAAAAAATAAAACGATCATGTGATCATCTGGGAA  
 TTGTCTGCCTAGATCACATCATCGTTGGAAAAAATAAATATTATAGTTTTC  
 GAGAAGAAGCAGATATTTTATAA

MYHIELKKEALLPRERLVDLGADRLSNQELLAILLRTGIKEKPVLEISTQILENI  
 SSLADFGQLSLQELQSIKGIGQVKSVEIKAMLELAKRIHKA EYDRKEQILSSEQ  
 LARKMMLELGDKKQEHLVAIYMDTQNR IIEQRTIFIGTVRRSVAEPREILHYAC  
 KNMATSLIIHNHPSGSPNPSESDLSFTKKIKRSCDHLGIVCLDHIIVGKNKYYSF  
 REEADIL\*

## Sequence description:

A] Length: 681 bp - 227 aa (full-length gene)

B] This gene sequence was not identified using the LEEP system. It was identified downstream of the ID-120 gene which was identified by LEEP,

FIG. 1 CONT'D

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during cloning and sequence analysis of the full-length ID-120 gene sequence.  
ATG start codon is preceded by an typical  
Shine-Dalgarno sequence. No obvious leader  
peptide sequence

ID-183

Clone 3-11b (ID-121b)

TGGTTAAAAGTAGTGATAGCTTGTATTCCATCTATTTTAATTGCTTTACCAT  
TTGATAATTGGTTTGAAGCTCATTTTAATTTTCATGATTCCGATTGCAATAGC  
CCTAATCTTTTATGGTTTTGTCTTCATATGGGTTGAAAAACGTAATGCACAC  
CTCAAACACAGGTAACCGAATTGGCAAGTATGTCTTACAAGACAGCTTTC  
TTGATTGGATGTTTCCAGGTTCTCAGTATTGTTCCGGGAACCAGTCGTTCTG  
GAGCTACTATTTTAGGAGCAATTATTATTGGAAGTAGTCGTTCCGGTCGCTG  
CTGACTTTACTTTCTTCCTTGCCATCCCAACTATGTTTGGTTATAGTGGACT  
TAAGGCGGTAAATATTTTTTAGATGGTAACGTCTTGAGTTTAGACCAATC  
TTTAATACTTTTAGTAGCAAGTCTGACAGCTTTCGTAGTTAGTTTATATGTT  
ATTCGTTTCTTGACAGACTATGTCAAACGACACGATTTACCATCTTTGGT  
AAGTATCGTATAGTCTTAGGAAGTTTACTCATCCTCTACTGGTTAGTTGTTT  
ATTTATTCTAA

WLKVVIACIPSILIALPFDNWFEAHFNFMIPAIALIFYGFVFIWVEKRNAHLKP  
QVTELASMSYKTAFLIGCFQVLSIVPGTSRSGATILGAIIGTSRSVAADFTFFLA  
IPTMFGYSLKAVKYFLDGNVLSLDQSLILLVASLTAFFVVSLEYVIRFLTDYVKR  
HDFTIFGKYRIVLGSLLILYWLVVHLF\*

Sequence description:

- A] Length: 579 bp - 193 aa (partial sequence)
- B] This gene sequence was not identified using the LEEP system. It was identified downstream of the ID-68 gene which was identified by LEEP, during cloning and sequence analysis of the full-length ID-68 gene sequence described in WO 00/06736. N-terminus has yet to be determined.

ID-184

Clone 3-11c (ID-121c)

**FIG. 1** CONT'D

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ATGGAAATGAAACAAATCAGTGAAACAACACTGAAAATTACAATTAGTAT  
 GGAAGATTTAGAAAGATCGTGGTATGGAGCTGAAAGATTTCTAATCCCTCA  
 GGAGAAGACTGAGGAATTTTTCTATTCTGTCATGGATGAATTAGACTTGCC  
 AGAAAACTTTAAAAATAGTGGTATGTTAAGTTTTTCGAGTAACACCTAAAA  
 AAGATCGCATTGATGTTTTTGTACAAAGTCTGAATTAAGTAAAGATTTAA  
 ATTTAGAAGAATTAGCAGATTTGGGTGACATTTCAAAAATGTCTCCAGAAG  
 ACTTTTTTAAAACCTTGGAACAATCGATGTTGGAAAAAGGGGATACGGAT  
 GCCCATGCCAAATTAGCAGAAATTGAAAATATGATGGATAAAGCAACTCA  
 AGAAGTAGTTGAGGAAAATGTTTCTGAAGAACAACCTGAAAAGGAAGTAG  
 AAACGATTGGATATGTTCACTATGTCTTTGATTTTGATAATATTGAAGCTGT  
 AGTTCGATTTTCACAAACGATTGATTTTCCAATAGAAGCTT

MEMKQISETTLKITISMEDLEDGRGMELKDFLIPQEKTEEFFYSVMDELDLPENF  
 KNSGMLSFRVTPKKDRIDVFVTKSELKDLNLEELADLGDISKMSPEDFFKTLE  
 QSMLEKGDTHAKLA EIENMMDKATQEVVEENVSEEQPEKEVETIGYVHY  
 VFDFDNIEAVVRFSQTIDFPIEA

#### Sequence description:

- A] Length: 547 bp - 182 aa (Partial sequence)
- B] This gene sequence was not identified using the LEEP system. It was identified downstream of the ID-68 gene which was identified by LEEP, during cloning and sequence analysis of the full-length ID-68 gene sequence. ATG start codon is preceded by an typical Shine-Dalgarno sequence. No obvious potential leader peptide sequence

ID-185

Clone 3-16b (ID-122b)

GGAAACCAACGGCCAGTACAATCGTCAAGGGTAGATTATCCTAAACGTAG  
 TCGTGCCAAGATTGTAGAAGTTTATTTTAGACAAGCTTCTACTACTGATTA  
 TTCTGGTGTTTACAAAGGTTACTATATTGACTTTGAAGCCAAAGAAACCCG  
 GCAGAAAACCTGCTATGCCTATGAAAAATTTTCATGCTCACCAAATAGAGC  
 ACATGGCAAATGTATTACAGCAAAAAGGGATTGCTTTGTCTTGCTTCATT

## FIG. 1<sub>CONT'D</sub>

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TTTCCACACTTAAGGAAACCTATCTACTCCCTGCTAATGAGTTAATTTTCATT  
 TTATCAGATTGATAAAGGCAATAAATCAATGCCTATTGATTATATCAGAAA  
 AAATGGATTTTTCGTAAAGGAGAGTGCCTTTCCTCAAGTCCCTTACTTAGA  
 TATTATTGAAGAAAAATTATTAGGCGGTGATTACAATTAA

GNQRPVQSSRVDYPKRSRAKIVEVYFRQASTTDYSGVYKGYIDFEAKETRQ  
 KTAMPMKNFHAHQIEHMANVLQQKGICFVLLHFSTLKETYLLPANELISFYQI  
 DKGNKSMPIDYIRKNGFFVKESAFPQVPYLDIIEEKLLGGDYN\*

#### Sequence description:

- A] Length: 447 bp - 149 aa (partial sequence)  
 B] This gene sequence was not identified using the LEEP system. It was identified upstream of the ID-122 gene which was identified by LEEP, during cloning and sequence analysis of the full-length ID-122 gene sequence. N-terminus has yet to be determined

ID-186

Clone 3-17b (ID-123b)

GGATCCTAAAAACGCTAAGGTTTATCAAAAAAATGCTGATCAATTTAGTG  
 ACAAGGCAATGGCTATTGCAGAGAAGTATAAGCCAAAATTTAAAGCTGCA  
 AAGTCTAAATACTTTGTGACTTCACATACAGCATTCTCATACTTAGCTAAG  
 CGATACGGATTGACTCAGTTAGGTATTGCAGGTGTCTCAACCGAGCAAGA  
 ACCTAGTGCTAAAAAATTAGCCGAAATTCAGGAGTTTGTGAAAACATATA  
 AGGTTAAGACTATTTTGTGGAAGAAGGAGTCTCACCTAAATTAGCTCAAG  
 CAGTAGCTTCAGCTACTCGAGTTAAAATTGCAAGTTTAAGTCCTTTAGAAG  
 CAGTTCCCAAAAACAATAAAGATTACTTAGAAAATTTGGAACTAATCTTA  
 AGGTACTTGTCAAATCGTTAAATCAATAG

DPKNAKVYQKNADQFSDKAMAIAEKYKPKFKAASKYFVTSHTAFSYLAKR  
 YGLTQLGIAGVSTEQEPSAKKLAIEIQEFVKTYKVKTFVEEGVSPKLAQAVAS  
 ATRVKIASLSPLEAVPKNNKDYLENLETNLKVLVKSLNQ\*

#### Sequence description:

# FIG. 1<sub>CONT'D</sub>

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A] Length: 433 bp - 144 aa (partial sequence)

B] This gene sequence was not identified using the LEEP system. It was identified downstream of the ID-123 gene which was identified by LEEP, during cloning and sequence analysis of the full-length ID-123 gene sequence. N-terminus has yet to be determined

ID-187

Clone 3-46/47 (ID-130b)

ATGAAAAAAGTCATCGATTTAAAAAACTACAAAAAGCATACGCCTCAGA  
 AACTGTTTTAAATAATATTAATTTGGAGGTGTTAAAGGAGAAATAATTGG  
 ATTAATAGGACCCTCTGGAGCAGGGAAATCTACCTTGATTAAACTATGCT  
 TGGCATGGAAAAAGCAGATAAGGGAACAGCTCTTGTTCTTGATACTCAAA  
 TGCCAGATCGTAATATTTTAAATCAAATTGGCTATATGGCTCAATCTGATG  
 CCTTACACGAGTCTTTAACTGGCTTAGAAAATTTATTATTCTTTGGAAAAA  
 TGAAAGGTATTCAAAAAACTGAATTAACAGCAGATAACTCATATTTCT  
 AAAGTAGTAGATCTAGAAAACCAACTTGATAAATTTGTCTCAGGTTACTCA  
 GAAGGTATGAAAAGACGGCTTTCTCTAGCCATCGCCCTACTTGGAACCCC  
 ACAGTTTTAATCCTAGATGAACCTACCGTTGGAATTGATCCATCCTTGAGG  
 AGAAAAATCTGGCAAGAGCTAATTAATATTAAGGATGAAGGACGTTCTAT  
 CTTTATTACAACCCACGTTATGGATGAAGCAGAATTAACAAGTAAGGTTGC  
 ACTACTATTACGTGGAAACATTATTGCCTTTGATACTCCATTACATTTAAA  
 AAAACAATTTAATGTGAGTACTATTGAGGAAGTTTTCTTAAAAGCTGAAGG  
 AGAATAA

MKKVIDLKKLQKAYASETVLNNINLEVFKGEIIGLIGPSGAGKSTLIKTMLGME  
 KADKGTALVLDTQMPDRNILNQIGYMAQSDALHESLTGLENLLFFGKMKGIO  
 KTELKQQITHISKVVDLENQLDKFVSGYSEGMKRRRLSLAIALLGNTVLILDEP  
 TVGIDPSLRRKIWQELINIKDEGRSIFITTHVMDEAELTSKVALLLRGNIIAFDTP  
 LHLKKQFNVSTIEEVFLKAEGE\*

Sequence description:

A] Length: 717 bp - 239 aa (Possible full-length sequence)

B] This gene sequence was not identified using the LEEP system. It was identified upstream of the ID-130 gene which was identified by LEEP, during cloning and sequence analysis of the full-length ID-130 gene sequence. ATG start codon is preceded by a possible

FIG. 1<sup>CONT'D</sup>

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Shine-Dalgarno. No obvious potential leader  
peptide sequence

ID-188

Clone 3-83b (ID-144b)

ATGGTACAAATGATACATGATATGATTAAAACAATTGAGCATTGCTGAG  
ACACAAGCTGATTTCCAGTGTATGATATTTTAGGGGAAGTCCATACTTAT  
GGACAACCTTAAAGTAGACTCTGACTCTCTAGCTGCTCATATTGATAGCCTA  
GGCCTTGTTGAAAAATCACCTGTCTTAGTATTCGGTGGTCAAGAATATGAA  
ATGTTGGCGACATTTGTTGCTTTAACAAAGTCAGGGCATGCTTATATACCG  
GTTGACCAACACTCTGCTTTGGATAGAATACAGGCTATTATGACAGTTGCT  
CAACCAAGCCTTATCATTTC AATTGGTGAATTCCTCTTGAAGTTGATAAT  
GTCCCAATCCTAGACGTTTCTCAAGTTTCAGCTATTTTTGAAGAAAAGACT  
CCTTATGAGGTAACACATTCTGTTAAAGGTGATGATAATTACTATATTATT  
TTCACCTCAGGGACTACTGTTTACCAAAGGTGTGCAAATTCACATGAC  
AATTTATTGAGCTTTACAAATTGGATGATTTCTGATGATGAGTTTTTCAGTTC  
CTGAAAGACCGCAAATGTTGGCTCAACCC

MVQMIHDMIKTIEHFAETQADFPVYDILGEVHTYGQLKVDSDSLAAHIDSLGL  
VEKSPVLVFGGQEYEMLATFVALTKSGHAYIPVDQHSALDRIQAIMTVAQPSL  
IISIGEFPLEVDNVPILDVSQVSAIFEKTPYEVTHSVKGDDNYIIFTSGTTGLP  
KGVQISHDNLLSFTNWMISDDEFSVPERPQMLAQP

Sequence description:

A] Length: 592 bp - 197 aa (partial sequence)

B] This gene sequence was not identified using the LEEP system. It was  
identified downstream of the ID-144 gene which was identified by LEEP,  
during cloning and sequence analysis of the full-length ID-144 gene sequence.Putative ATG start codon is preceded by a  
typical Shine-Dalgarno sequence. No obvious  
leader peptide sequence

This orf is not in frame with nuc

ID-189

FIG. 1<sub>CONT'D</sub>

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Clone 3-86b (ID-145b)

ATGGAAAATCATCGTTATGAAGATGAAGGTAAATTCCAGCGTAAGATGAC  
CAGTCGTCATCTCTTTATGTTATCGCTAGGTGGTGTATCGGGACTGGGCTT  
TTCTTGAGTTCAGGTTATACCATTCACAGGCTGGTCCGCTTGGAGCTGTG  
CTGTCTTATTTGATTGGTGCCGTTGTGGTTTATTTGGTCATGCTATCACTTG  
GGGAATTGGCGGTTGCCATGCCGGTGACGGGGTCATTCCACACTTATGCCA  
CTAAGTTTATCAGTCCTGGAACAGGTTTTACTGTTGCTTGGCTATATTGGAT  
TTGTTGGACGGTCGCCTTGGGGACTGAATTTTTAGGTGCTGCCATGCTGAT  
GCAGCGCTGGTTCCCAAATGTGCCGGCTTGGGCATTTGCTTCCTTTTTTGGC  
CTTGTGATTTTTGGTTTAAATGCTCTTAGCGTACGCTTTTTTGCAGAAGCAG  
AGTCTTTCTTCTCAAGTATTAAGGTTATTGCTATCATTATCTTTATTATCTTG  
GGCTTAGGTGCTATGTTTGGTCTAGTTTCCTTTGAAGGTCAGCACAAGGCT  
ATTCTCTTCACTCATCTGACTGCCAATGGTGCCTTTCCAAATGGTATCGTTG  
CAGTTGTCTCAGTCATGTTGGCTGTAACTATGCCTTCTCTGGTACTGAGTT  
AATTGGTATTGCGGCTGGTGAAACGGATAATCCCAAAGAAGCTGTACCAA  
GGGCTATTAAACGACAATCGGTGCTTGGTTGTTTTCTTTGTACTGACAA  
TTGTTGTCCTAGCTTCGCTATTGCCAATGAAAGAGGCAGGCGTATCCACAG  
CACCATTCTGTTGATGTCTTTGACAAGATGGGAATCCCTTTTACGGCGGATA  
TCATGAACCTTCGTTATCTTGACAGCCATCCTGTCTGCTGGTAACTCAGGTCT  
CTACGCATCAAGCCGTATGCTCTGGTCCCTTGCCAATGAAGGTATGTTGTC  
AAAATCTGTTGTGAAAATCAATAAACACGGTGTCCCAATGCGTGCTCTTCT  
CTTGTCAATGGCAGGAGCAGTGCTGTGCTCTTTTCAAGTATTTACGCTGC  
AGACACAGTTTATCTAGCCTTGGTTTCAATCGCGGGCTTTGCTGTTGTTGTC  
GTATGGCTAGCCATTCCAGTCGCACAAATCAATTTCCGCAAGGAATTC

MENHRYEDEGKFQRKMTSRHLFMLS LGGVIGTGLFLSSGYTIAQAGPLGAVL  
SYLIGAVVVYLVMLSLGELAVAMPVTGSFHTYATKFISPGTGFTVAWLYWIC  
WTVALGTEFLGAAMLQMQRWFPNVPAPAFASFFALVIFGLNALSVRFFAEAES  
FFSSIKVIAIIIFILGLGAMFGLVSFEGQHKAILFTHLTANGAFPNGIVAVVSVM  
LAVNYAFSGTELIGIAAGETDNPKEAVPRAIKTTIGRLVVFVLTIVVLASLLPM  
KEAGVSTAPFVDVFDKMGIPFTADIMNFVILTILSAGNSGLYASSRMLWSLA  
NEGMLSKSVVKINKHGVPMRALLSMAGAVLSLFSSIYAADTVYLALVSIAGF  
AVVVVWLAIPVAQINFRKEF

Sequence description:

A] Length: 1126 bp - 393 aa (partial gene)

**FIG. 1** CONT'D

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sequence)

B] This gene sequence was not identified using the LEEP system. It was identified downstream of the ID-145 gene which was identified by LEEP, during cloning and sequence analysis of the full-length ID-145 gene sequence. Putative ATG start codon is preceded by a typical Shine-Dalgarno sequence. Possesses a possible leader peptide sequence.

ID-190

Clone 3-94b

TCAGAAAATGCAGAGGCAGCAACGGTTGCCACAACTTGGTTACCAAAGG  
 AGCTAATGTCATTATCGGACCAGCAACATCGGGTGCAGCTGCATCTTCAAC  
 TCCAAAAGTAAATGCAGCAGCAGTTCCAATGATTGCACCTGCTGCGACAC  
 AAGACAATTTAGTCTATGGTTCTGATGGAAAAACCTTAAATCAGTATTTCT  
 TCCGAGCTACTTTTGTGCGATAATTATCAAGGAAAGCTATTGTCTCAGTATG  
 CTACAGACAACCTTAAAGCTAAAAAAGTTGTTCTATTTTATGATAATTCAT  
 CAGATTACTCAAAGGGGGTAGCAAAATCATTTAAGGAAAGTTATAGTGGA  
 AAAATTGTTGATAGTATGACATTCTCCGCTGGTGATACTGATTTCCAAGCG  
 TCATTGACTAAGTTGAAAGGGGAAAGAATATGATGCTATTGTGATGCCAGG  
 TTACTATACCGAGACAGGATTAATAGTTAAGCAAGCGCGTGATTTAGGTAT  
 CTCTAAACCGGTTCTTGGGCCTGATGGTTTGGATAGTCCGAAATTTGTGCA  
 ATCGGCAACACCTGTAGGAGCTTCAAACGTTTATTATTTGACAGGTTTCAC  
 TACACAAGGATCAACCAAAGCTAAAGCT

SENAEAATVATNLVTKGANVIIGPATSGAAASSTPKVNAAAVPMIAPAATQD  
 NLVYGS DGKTLNQYFFRATFVDNYQGKLLSQYATDNLKAKKVLFYDNSSD  
 YSKGVAKSFKESYSGKIVDSMTFSAGDTDFQASLTKLKGKEYDAIVMPGYT  
 ETGLIVKQARDLGISKPVLPDGFDSPKFVQSATPVGASNVYYLTGFTTQGST  
 KAKA

Sequence description

A] Length: 637 bp - 231 aa (partial sequence)

FIG. 1 CONT'D

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B] This gene sequence was not identified using the LEEP system. It was identified upstream of the ID-149 gene which was identified by LEEP, during cloning and sequence analysis of the full-length ID-149 gene sequence. N- and C-termini have yet to be determined

ID-191

Clone 2-c94b (ID-153b)

TTGGGACTTAAAGACCATGCTTTAGTCTATCCATTTTCATTATCTGGGGGG  
CAAAAGCAACGTGTGCGCACTAGCTCGTGCGATGATGATTGATCCACAGATT  
ATTGTTTATGATGAGCCAAGTACGCTCTTGATCCAGAGTTGCGTCAAGAA  
GTAGAAAACTAATTTTACAAAATAGAGAAACAGGTATGACACAAATTGT  
AGTAACACATGATCTTCAATTTGCTGAAAGTATATCTGATACGATTCTCAA  
AATTAATCCTAAGTAG

MGLKDHALVYPFSLSGGQKQRVALARAMMIDPQIIGYDEPTSALDPELRQEV  
EKLILQNRETGMTQIVVTHDLQFAESISDTILKINPK\*

Sequence description

A] Length: 270 bp - 90 aa (partial sequence)

B] This gene sequence was not identified using the LEEP system. It was identified upstream of the ID-153 gene which was identified by LEEP, during cloning and sequence analysis of the ID-153 gene sequence.  
N-terminus has yet to be determined

ID-192

Clone 2-c1b (ID-155b)

ATGACTAATATCTCAGATGTTCCAAAAGCTATTAGAACACAGGCACAGTAT  
GTTCTCTTGGGAATGAGAGTTATGGATCAGTCGGTATTACCGAAAACATAT  
AATTCAAAGAACCTTATTTGAAACCAGATATGATTTATATTCATGATAGA

**FIG. 1** CONT'D

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AGACAAGAGACAATGCTTAAAATCACTCAAGAAATAGAAATGGAGCATTG  
A

MTNISDVPKAIRTQAQYVLLGMRVMDQSVLPKTYNSKEPYLKPDMIYIHDRR  
QETMLKITQEIEMEH\*

#### Sequence description

A] Length: 204 bp - 68 aa (partial sequence)

B] This gene sequence was not identified using the LEEP system. It was identified upstream of the ID-155 gene which was identified by LEEP, during cloning and sequence analysis of the ID-155 gene sequence.

ATG start codon is preceded by a potential typical Shine-Dalgarno sequence.

Has a

typical leader peptide. N-terminus has yet to be verified

ID-193

Clone 2-54altb (ID-172b)

AAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCTTGGGGAATATAAATT  
TGGATTTTCATGACGATGTAAAGCCAATTTATTCTACGGGAAAAGGTCTAAA  
TGAGGCTGTTATTCGTGAGTTATCTGCAGCTAAGGGTGAACCTGAGTGGAT  
GTTGGACTTTCGTCTAAAATCCTTGGAAACGTTTAATAAAAATGCCGATGCA  
GACCTGGGGAGCAGATTTATCAGATATTGATTTTGATGATATTATTTATTA  
TCAAAAAGCATCTGATAAACCTGCGCGTGATTGGGATGATGTTCCAGAAA  
AAATCAAAGAACTTTTGAAAGAATTGGGATTCCAGAAGCTGAAAGAGCC  
TATCTTGCAGGAGCATCAGCACAATATGAATCAGAAGTAGTTTATCACAAT  
ATGAAAGAAGAATATGATAAGCTGGGTATTGTTTTTACGGATACTGACTCT  
GCACTTAAAGAGTACCCAGAGCTATTCAAAAAATATTTTGCTAAACTTGTC  
CCTCCAACAGATAATAAATTAGCTGCTCTGAACTCTGCTGTATGGTCAGGT  
GGAACATTTATTTATGTTCTTAAAGGTGTTAAGGTGGATATTCCACTTCAA  
ACTTACTTCCGTATTAATAATGAAAATACTGGACAATTTGAACGTACTCTC  
ATTATTGTTGATGAGGGAGCAAGTGTTCACTATGTTGAAGGTTGTACCGCC  
CCAACCTATTCTTCAAATAGTTTACATGCAGCTATAGTTGAAATTTTTGCAC  
TTGATGGAGCTTATATGCGCTATACGACTATTCAAAATTGGTCCGATAATG  
TCTATAATTTAGTGACAAAACGTGCTACCGCTAAAAAAGATGCAACAGTT  
GAGTGGATAGATGGAAATCTAGGAGCTAAAACAACAATGAAATACCCATC

FIG. 1<sub>CONT'D</sub>

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GGTTTACCTTGATGGTGAAGGAGCACGTGGCACGATGTTGTCTATTGCTTT  
 TGCAAACAAAGGACAACACCAAGATACGGGTGCAAAGATGATTCATAATG  
 CCCCCATACTAGTTCATCCATTGTCTCTAAATCAATTGCTAAGGGTGGGG  
 GAAAAGTTGATTATCGAGGTCAAGTGACATTTAATAAAGATTCCAAAAA  
 TCAGTGTACATATAGAATGTGACACCATATTGATGGATGATATTTCAAAA  
 TCAGATACCATAACCGTTTAATGAGATTCATAATTCACAGGTTGCTTTAGAG  
 CATGAAGCAAAGGTGTCTAAGATTTCTGAAGAGCAACTGTACTACTTGATG  
 AGTCGAGGTTTATCTGAAGCTGAAGCAACAGAAATGATTGTTATGGGGTTT  
 GTTGAGCCCTTTACGAAAGAATTACCAATGGAATATGCGGTAGAGTTAAA  
 TCGTTTAATTTCTATGAAATGGAAGGTTTCAAGTTGGTTAA

MHACRSTLEDLGEYKFGFHDDVKPIYSTGKGLNEAVIRELSAAKGEPEWMLD  
 FRLKSLETFNKMPMQTWGADLSIDFDDIIYYQKASDKPARDWDDVPEKIKE  
 TFERIGIPEAERAYLAGASAQYSEVVYHNMKEEYDKLGIVFTDSDSALKEYP  
 ELFKKYFAKLVPPTDNKLAALNSAVWSGGTFIYVPKGVKVDIPLQTYFRINNE  
 NTGQFERTLIIVDEGASVHYVEGCTAPTYSSNSLHAAIVEIFALDGAYMRYTTI  
 QNWSDNVYNLVTKRATAKKDATVEWIDGNLGAKTTMKYPSVYLDGEGARG  
 TMLSIAFANKGQHQDTGAKMIHNAPHTSSSIVSKSIKGGGKVDYRGQVTFN  
 KDSKKS SVSHIECDTILMDDISKSDTIPFNEIHNSQVALEHEAKVSKISEEQLYYL  
 MSRGLSEAEATEMIVMGFVEPFTKELPMEYAVELNRLISYEMEGSVG\*

#### Sequence description:

- A] Length: 1411 bp - 469 aa (Possible full-length gene)
- B] This gene sequence was not identified using the LEEP system. It was identified downstream of the ID-72 gene which was identified by LEEP, during cloning and sequence analysis of the full-length ID-72 gene sequence. No obvious Shine Dalgarno sequence upstream of TTG start codon (insufficient sequence data). N terminus needs verification.

ID-194

Clone 3-1b (ID-81b)

ATGATAGAATTCTTTTCTAATATCAGAACAGAGATTCCGCAGATGCCTTTA  
 CTTATCCATAGTTTGATTTTATCTGTCTTACCTTTTCTGATGTGGCTGACTTT  
 GGTTAATAGAGATAAGCCTTTGTATAAACTATTTGGAGTATCCTTTTAGG  
 ACTTCAGTTAATTACGATTTATACTTGGTTTTTCTGGGCAAATTGCCTTTA

**FIG. 1** CONT'D

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TCTGAAAGTCTTCCCCTTTACCATTGTCGAATAGGCATGTTTGTCTGGTCTCT  
TA

MIEFFSNIRTEIPQMPLLIHSLILSVLPFLMWLTLVNRDKPLYKTIWSILLGLQLI  
TIYTWFFWAKLPLSESLPLYHCRIGMFVGLL

#### Sequence description

A) Length: 261 bp - 87 aa (partial gene sequence)

B) This gene sequence was not identified using the LEEP system. It was identified downstream of the ID-81 gene which was identified by LEEP, during cloning and sequence analysis of the full-length ID-81 gene sequence. Sequence Characteristics: Possesses a potential leader peptide sequence. Orf is preceded by a potential Shine-Dalgarno sequence.

ID-195

Clone RS-55b

AAGCTTGTGCAAAGTATTAAAGAGATAGGATTAGCTAATGCGCATTTATTA  
GCTGTTGCTCCGACAGGGTCAATCAGTTATCTTTCTTCTTGTACTCCGAGCC  
TTCAACCGGTTGTATCACCTGTCGAAGTACGCAAGGAAGGAGCACTGGGG  
AGGGTTTATGTAGCTGCTTATAAGATTGATGCAGATAATTATGTCTACTAC  
AAAAAAGGAGCTTATGAAGTGGGATCTGAGGCGATTATCAATATTGCAGC  
TGCCGCTCAAAAACACATTGATCAAGCTATTCGTTAACGCTTTTCATGAC  
AGATCAAGCAACTACGCGAGATTTAAATAAAGCCTATATTCAAGCATTTA  
AACAAAAATGTGCCTCTATTTATTATGTACGAGTGAGACAGGACATCCTAG  
AAGGTAGCGAGAGTTATGATGATATGCTGGATGATTTCACTTCATCGGACT  
TAGAAGACTGTCAATCCTGCATGATTAA

>KLVQSIKEIGLANAHLAVAPTGSISYLSSCTPSLQPVVSPVEVRKEGALGRV  
YVAAYKIDADNYVYYKKGAYEVGSEAIINIAAAAQKHIDQAISLTLFMTDQAT  
TRDLNKAYIQAFKQKCASIYYVRVRQDILEGSESYDDMLDDFTSSDLEDCQSC  
MI\*

Sequence description:

FIG. 1 CONT'D

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A] Length 486 bp - 162 aa (Partial sequence)

B] This gene sequence was not identified using the LEEP system. It was identified upstream of the ID-87 gene which was identified by LEEP, during cloning and sequence analysis of the full-length ID-87 gene sequence. N-terminus to be determined.

ID-196

Clone RS-59(ID-90b)

GTGAGGACATATATTACAAACTTGAATGGACATTCAATCACTAGTACAGC  
ACAAATAGCTCAAAACATGGTAACAGATATAGCAGTAAGCTTAGGTTTTC  
GTGAGCTGGGAATACATTCTTATCCGATTGATACTGATTCTCCTGAGGAAA  
TGAGTAAGCGTTTAGATGGAATCTGTTCCGGACTTAGAAAAAATGATATTG  
TCATATTTTCAGACACCTACATGGAACACTACAACCTTTTGATGAAAAATTAT  
TTCACAAATTAAAAATATTTGGTGTAAGATTGTTATTTTATACATGATGT  
TGTACCGCTAATGTTTGATGGAAATTTTATTTGATGGATAGAAGCTATAGC  
TTATTATAATGAAGCAGATGTTTAATAGCCCCTAGTCAAGCAATGGTCGAT  
AAGCTT

MRTYITNLNGHSITSTAQIAQNMVTDIAVSLGFRELGIHSYPIDTDSPEEMSKRL  
DGICSGLRKNDIVIFQTPTWNTTTFDEKLFHKLKIFGVKIVIFIHDVVPLMFDGN  
FYLMDRITIAYYNEADVLIAPSQAMVDKL

Sequence description:

A] Length: 414 bp - 138 aa(partial gene)

B] This gene sequence was not identified using the LEEP system. It was identified downstream of the ID-90 gene which was identified by LEEP, during cloning and sequence analysis of the full-length ID-90 gene sequence. No obvious signal peptide, but a possible Shine Dalgarno sequence is present upstream of ATG start codon. C-terminus has yet to be determined.

ID-197

Clone RS-59c (ID-90c)

FIG. 1 CONT'D

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CATGGAAATGAAGTTGATGATGTTATTAGAAGGGCATTGAATATAATCAC  
CTTATCTTTGCTTTTGATAATACCTGTCATAACAGAGAGTTAGTATTAGATA  
GCAATATCATTCTCACACAACCTGTGAACAATTGATAAATTTAATGAAAA  
ATTTATCAGGCTCCATTATGTATTTGCTAGAGCAACAAAGAGAACAAACA  
AGTAATGAAACAAAAGAGCGTTATAAAGAAATATTAGGAGGGTATGGAA  
ATGCCTAA

HGNEVDDVIRRAFEYNHLIFAFDNTCHNRELVLDSNIISHTTCEQLINLMKNLS  
GSIMYLLEQQREQTSNETKERYKEILGGYGNA\*

Sequence description:

A] Length: 261 bp - 87 aa (partial gene sequence)

B] This gene sequence was not identified using the LEEP system. It was identified upstream of the ID-90 gene which was identified by LEEP, during cloning and sequence analysis of the full-length ID-90 gene sequence. N-terminus has yet to be determined

ID-198

Clone RS-70b (ID-93b)

ACATTTTTATATTATGTATTTGAAGACGTAGCCACCCAGTCAAATATGACT  
GGGAAGATTTTAGTATGTCTAAAGAAGAGTTGTCATATTTACCCGTTATT  
AAACTTTTAAAGAATCAAGGTGTATACAACGGCTTGATTGGTCTATTCCTC  
CTTTATGGGTTATATATTTACAGAAATCAAGAAATTGTAGCTATTTTTTAA  
TCAATGTGTTGCTAGTTGCTGTTTATGGTGCTTTGACAGTTGATAAAAAAA  
TCTTATTAAACAGGGTGGTTACCTATATTAGCTCTTTTAACATTCTTATT  
TTAA

TFLYYVFEDVATQSNMTGKIFSMSKEELSYLPVIKLFKNQGVYNGLIGLFLLY  
GLYISQNQEIVAIFLINVLLVAVYGALTVDKILLKQGGLPILALLTFLF\*

Sequence description:

A] Length: 312 bp - 104 aa (partial gene sequence)

**FIG. 1** CONT'D

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B] This gene sequence was not identified using the LEEP system. It was identified upstream of the ID-93 gene which was identified by LEEP, during cloning and sequence analysis of the full-length ID-93 gene sequence.

N-terminus has yet to be determined

ID-199

Clone RS-70c (ID-93c)

ATGAAATTAAGTGTCTTGATTATGGGCTTATTGATTATGGAAAACTGCA  
AGTGATGCAATACAAGAAACGATTCTTTTATCACAAGAGGCGGAGCAACT  
AGGCTATCATCAATTTTGGGTGGCTGAACATCACGGTGTTAAGGCATTTCAG  
TATTAGCAATCCAGAATTAATGATAATGCATTTGGCTAACCAGACTAAATC  
TATCAAAATTGGCTCTGGAGGTATAATGCCTCTGCACTATAGTAGTTTTAA  
ACTCGCGGAGACTCTCAAGACATTAGAGACATGTCATCCGAATCGAGTAA  
GTATTGGTTTAGGAAATTCAGTAGGGACAGTTAAAGTTTCAAATGCACTTC  
GTAGCTTACATAAAGCACATGATTACGAAGAGGTACTGGAGGAATTGAAG  
TCATGGCTTATTGATGAATCATCCAGTAAGGAACCATTAGTTCAACCGACT  
CTTTCTAGCTTCCCAGACTTATATGTGTTGGGGAGTGGTCAAAAATCAGCT  
TATTTAGCGGCTAAACTTGGCTTAGGCTTTACCTTCGGTGTTTTTCCTTTTA  
TGGACAAAGACCCATTGACAGAAGCTAAA

MKLSVLDYGLIDYGKTASDAIQETILLSQEAEQLGYHQFWVAEHHGVKAFSIS  
NPELMIMHLANQTKSIKIGSGGIMPLHYSSFKLAETLKTLETCHPNRVSIGLGN  
SLGTVKVSNALRSLHKAHDYEEVLEELKSWLIDESSSKEPLVQPTLSSFPDLYV  
LGSGQKSAYLAAKLGLGFTFGVFPFMDKDPLTEAK

Sequence description:

A] Length: 588 bp - 196 aa (partial)

B] This gene sequence was not identified using the LEEP system. It was identified downstream of the ID-93 gene which was identified by LEEP, during cloning and sequence analysis of the full-length ID-93 gene sequence. No obvious signal peptide, but Shine Dalgarno sequence upstream of the ATG start codon.

FIG. 1 CONT'D

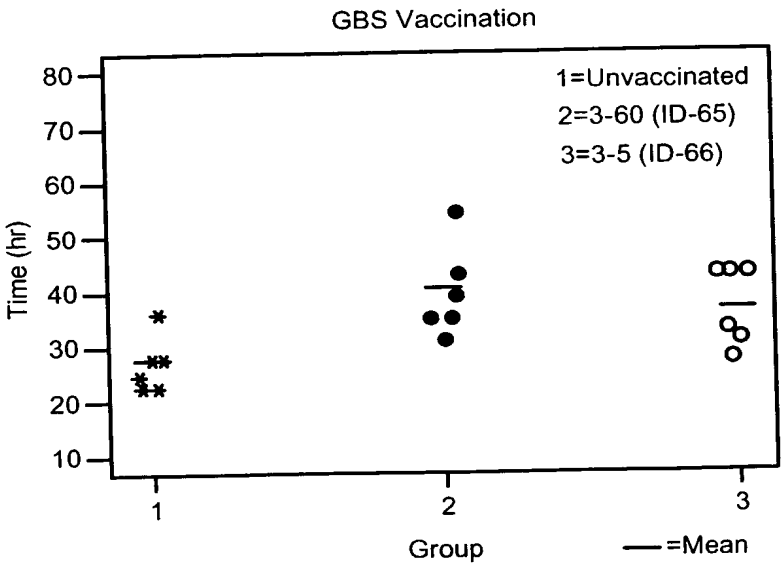


FIG. 2

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nucS1

Bgl II Eco RV

5'-cgagatctgatatctcacaacagataacggcgtaaataag -3'

nucS2

Bgl II Sma I

5'-gaagatcttccccgggatcacaacagataacggcgtaaataag -3'

nucS3

Bgl II Eco RV

5'-cgagatctgatatccatcacaacagataacggcgtaaataag -3'

nucR

Bam HI

5'-cgggatcccttatggacctgaatcagcggtgtgc -3'

NucSeq

5'-ggatgctttgtttcaggtgtatc -3'

pTREP<sub>F</sub>5'-catgatatcggtacctcaagctcatatcattgtccggcaatgggtgtgggctttttttgttttagcggataa  
caatttcacac -3'pTREP<sub>R</sub>5'-goggatcccccgggcttaattaatgtttaaacactagtcgaagatctcgcgaattctcctgtgtgaaatt  
gttatccgcta -3'pUC<sub>F</sub>

5'-cgccagggttttcccagtcacgac -3'

V<sub>R</sub>

5'-tcaggggggcgagacctatg -3'

V<sub>1</sub>

5'-tcgtatgttggtggaattgtg -3'

V<sub>2</sub>

5'-tccggctcgtatgttggtggaattg -3'

FIG. 3

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pTREP-Nuc vectors allow cloning of genomic DNA into each frame with respect to the nuclease gene

(i)

|                     |  |          |
|---------------------|--|----------|
| pTREP1-nuc1 (EcoRV) | AAGTATCAGATCT-- <u>GATATC</u> --TCACAAACAGATAACGGCGTAAAT | Frame=+1 |
|                     | .....▲.....  |          |
| pTREP1-nuc2 (Sma I) | AAGTATCAGATCTTCCCGGGA-TCACAAACAGATAACGGCGTAAAT           | Frame=+2 |
|                     | .....▲.....  |          |
| pTREP1-nuc3 (EcoRV) | AAGTATCAGATCT-- <u>GATATCCATC</u> ACAAACAGATAACGGCGTAAAT | Frame=+3 |
|                     | .....▲.....  |          |
| Nuclease Gene       | TCACAAACAGATAACGGCGTAAAT                                 |          |

Cloning site is indicated by an arrow

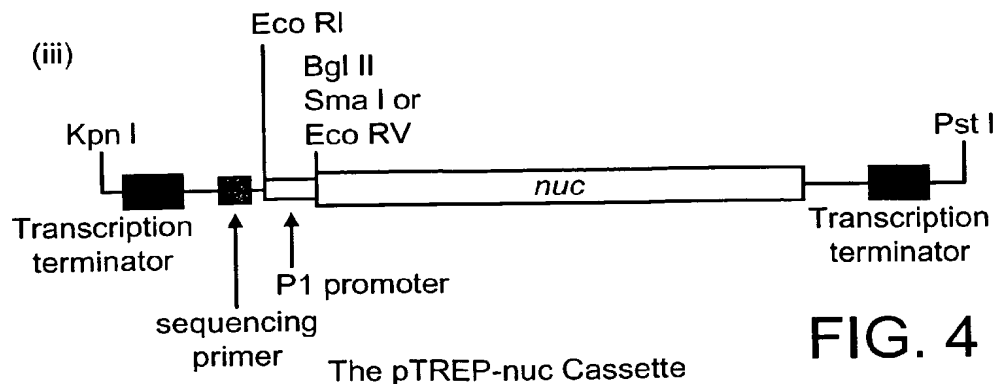
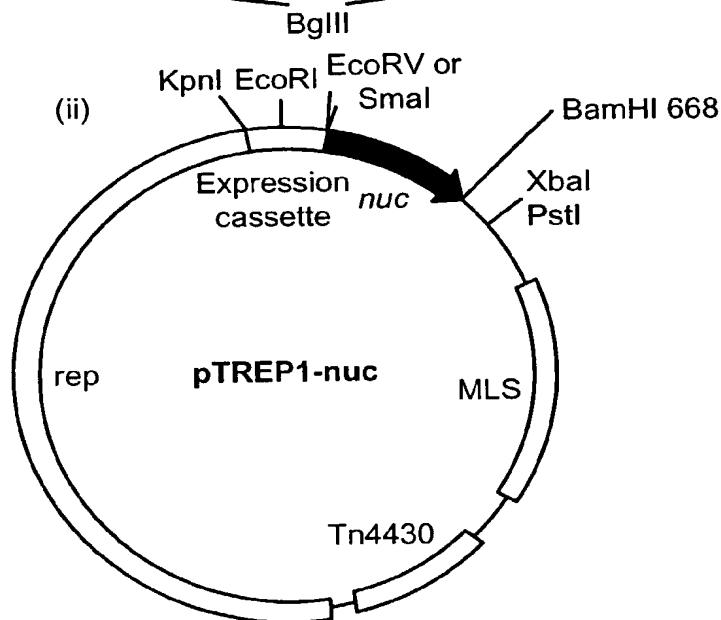
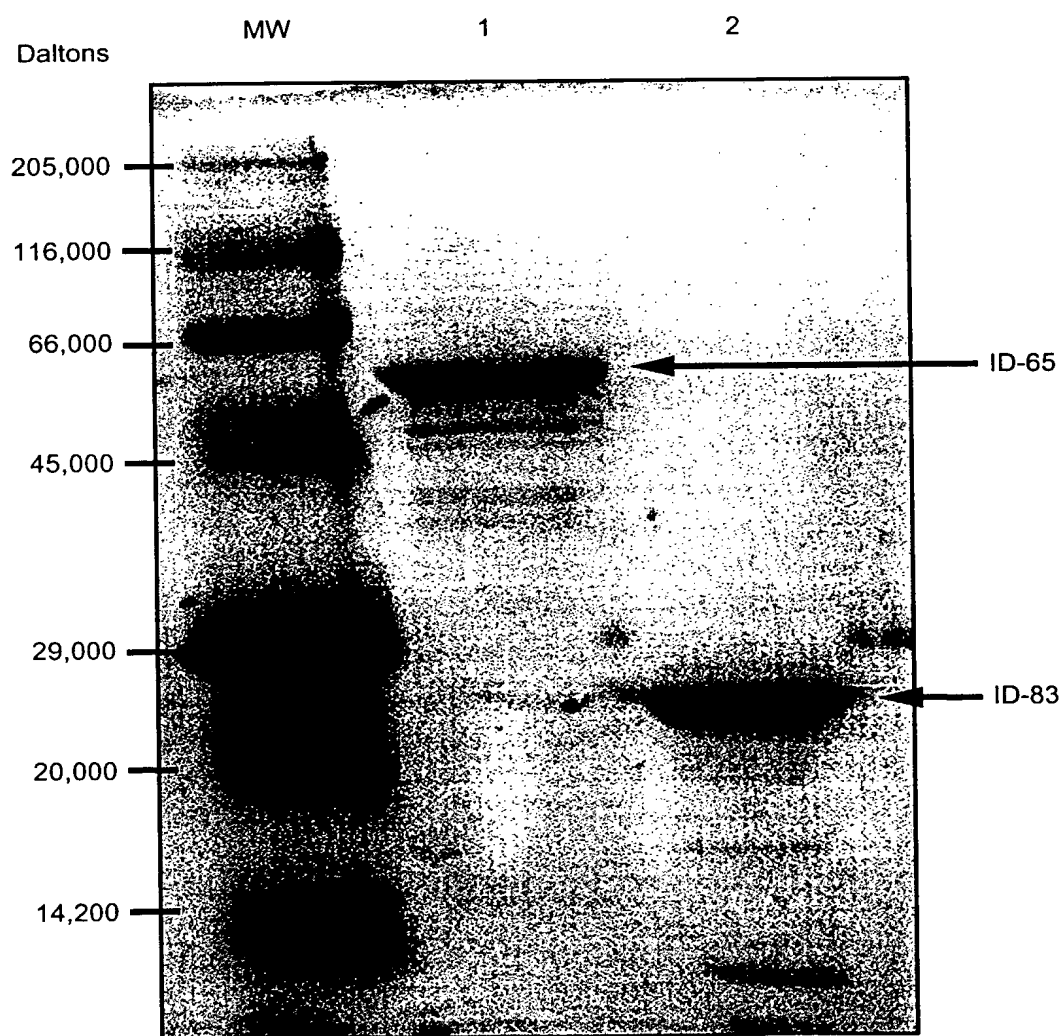


FIG. 4

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**FIG. 5**

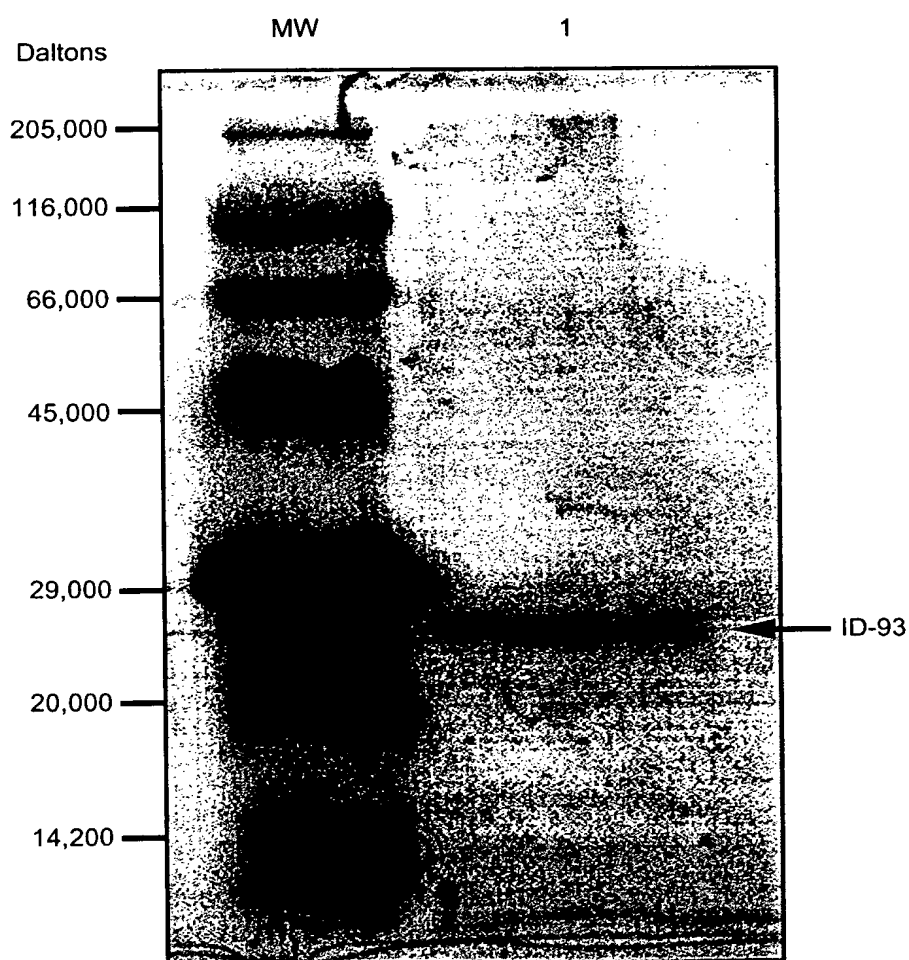
SDS-PAGE analysis of the purified ID-65 and ID-83 protein antigens



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**FIG. 6**

SDS-PAGE analysis of the purified ID-93 antigen



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**FIG. 7**

SDS-PAGE analysis of the purified ID-89 and ID-96 protein antigens

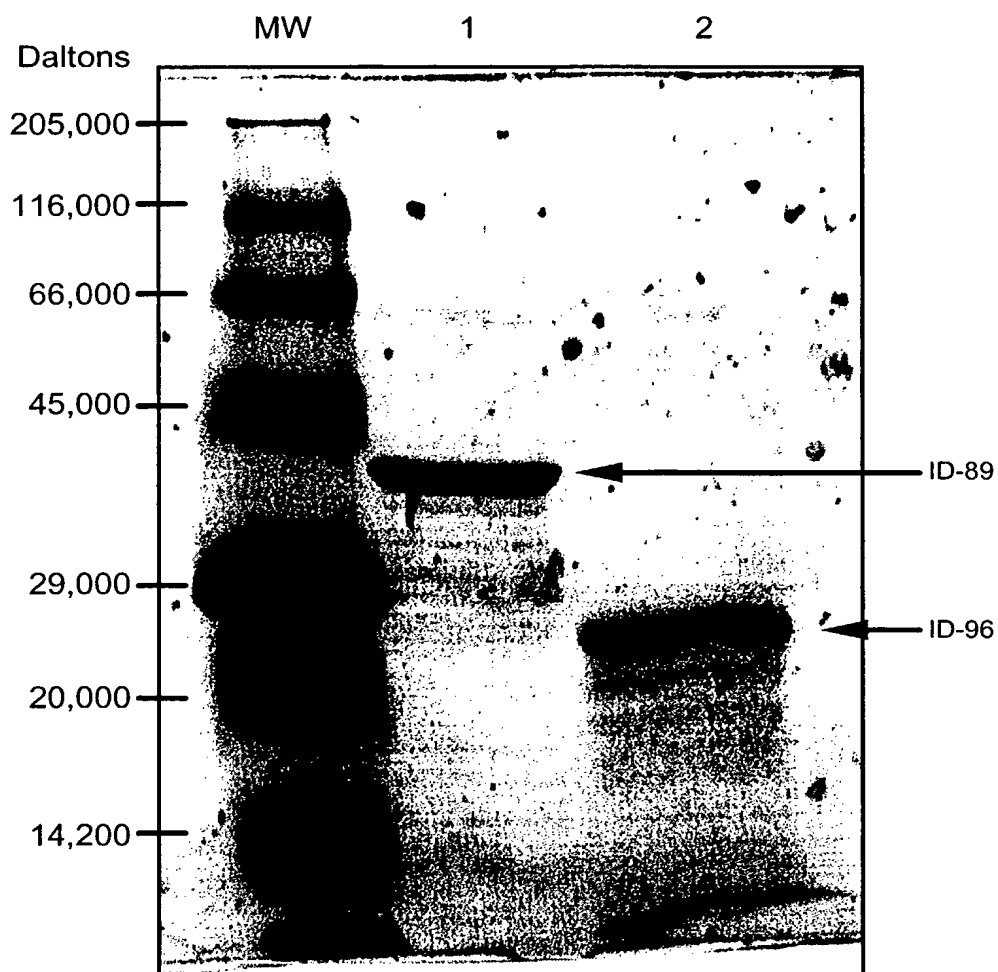


FIG. 8

IgG Titres against the ID-65 and ID-83 proteins

ID-65 and ID-83 Vaccinations -IgG Titres

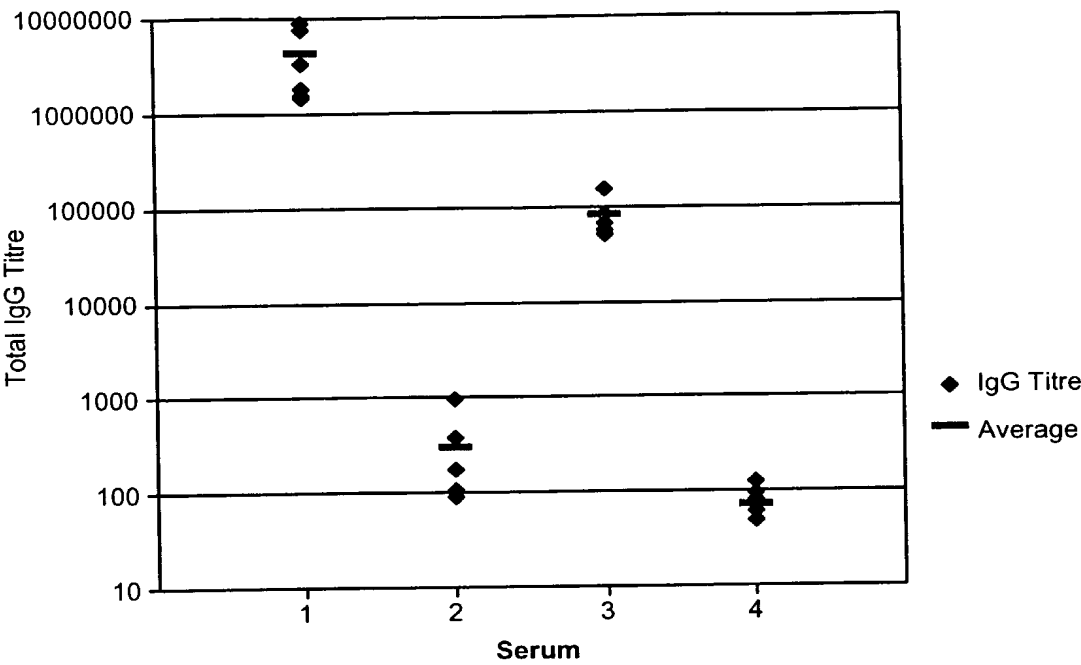
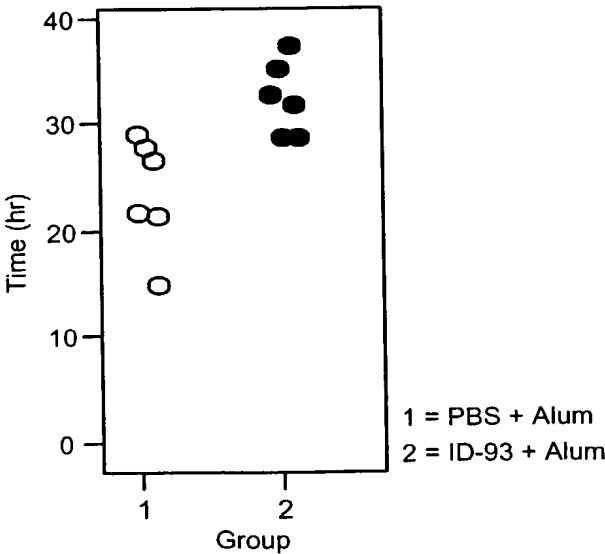


FIG. 9

Survival data

ID-93 Vaccination- GBS Challenge and Survival

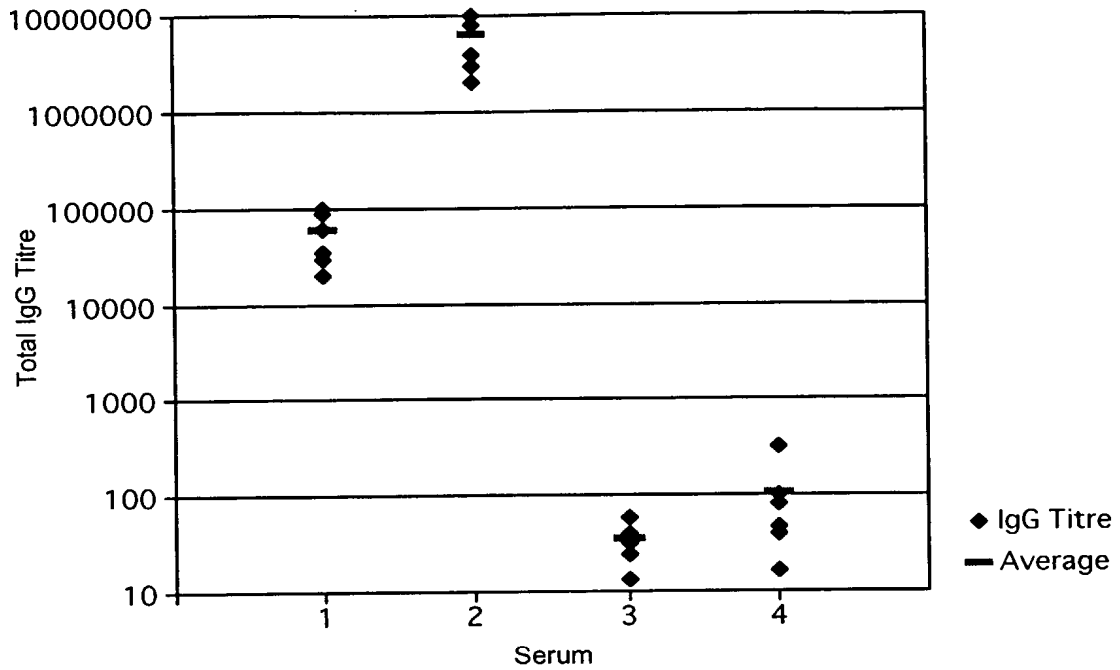


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# FIG. 10

IgG Titres against the ID-93 protein

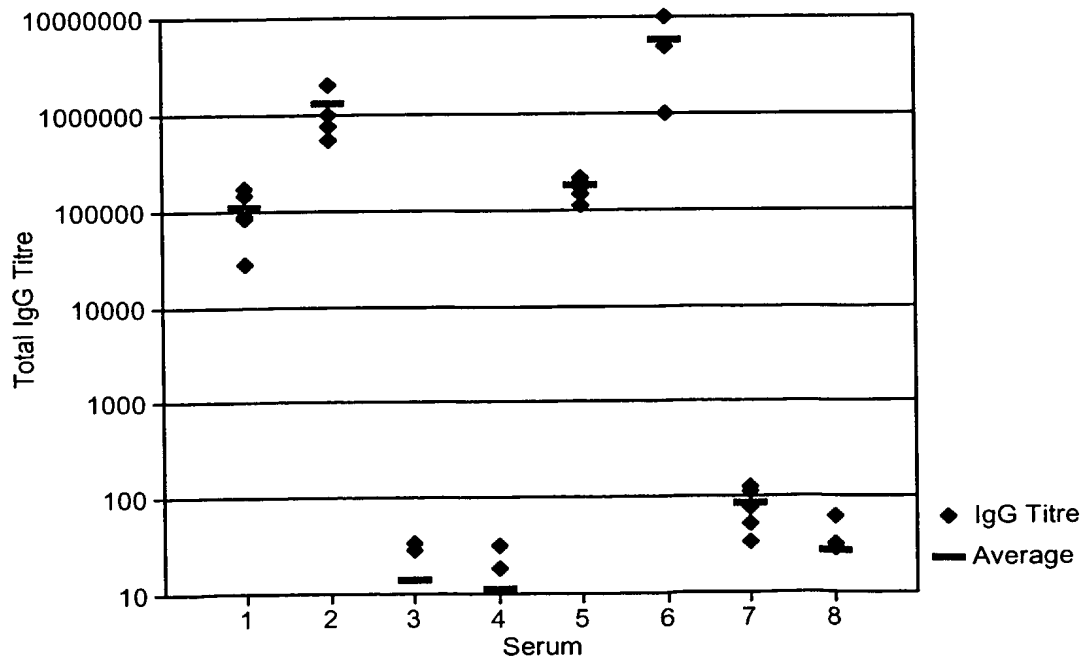
ID-93 Protein Vaccine -IgG Titres



# FIG. 11

IgG Titres against the ID-89 and ID-96 proteins

ID-89 and ID-96 Protein Vaccines -IgG Titres



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## FIG. 12

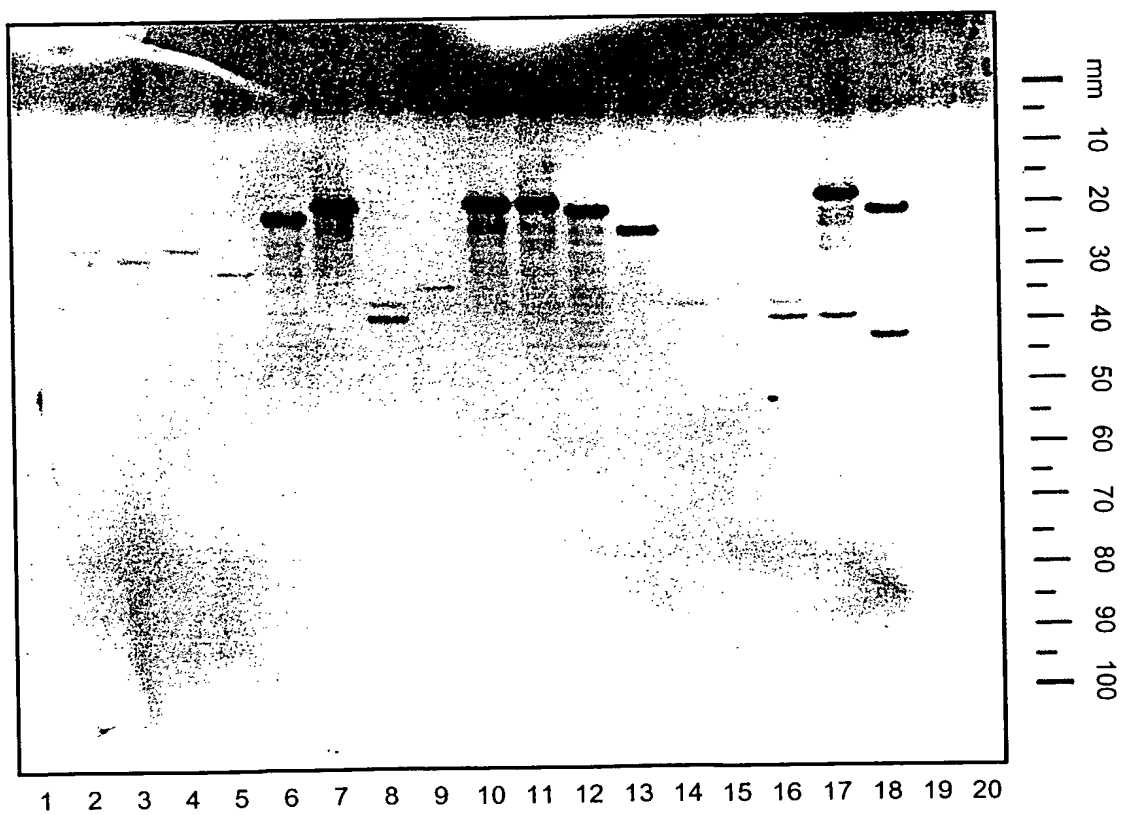
Southern blot analysis - *rib*

FIG. 13

Southern blot analysis - ID-65

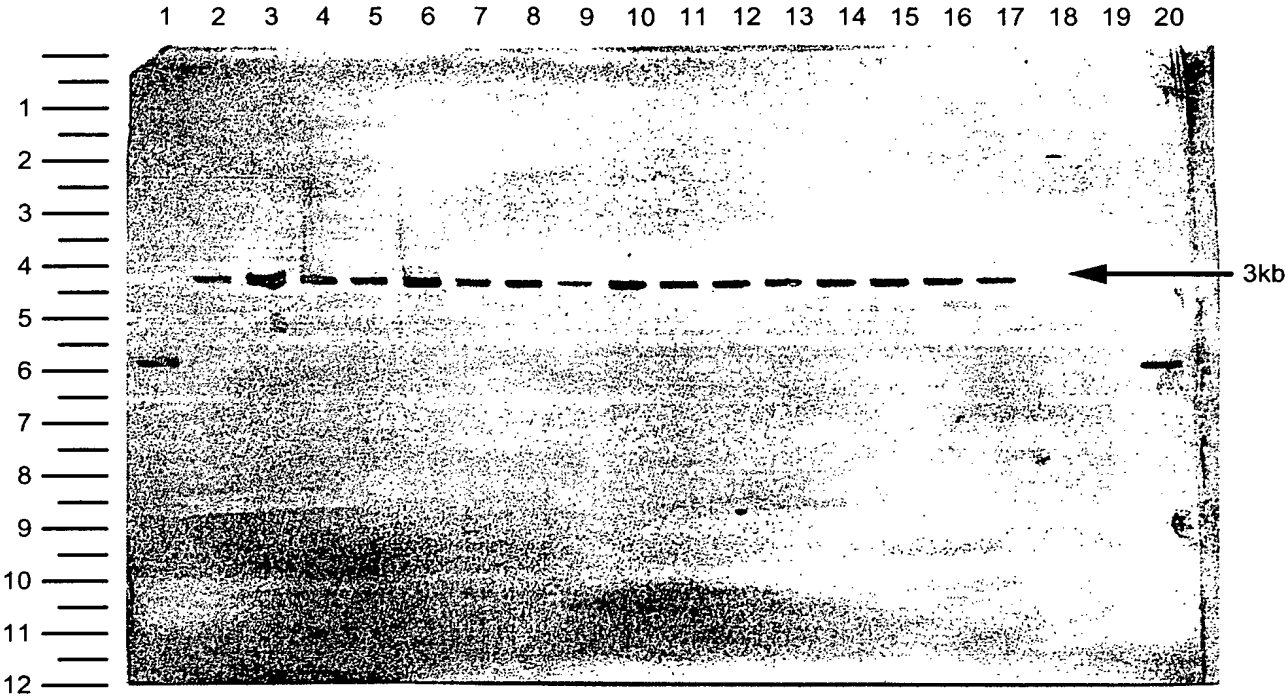
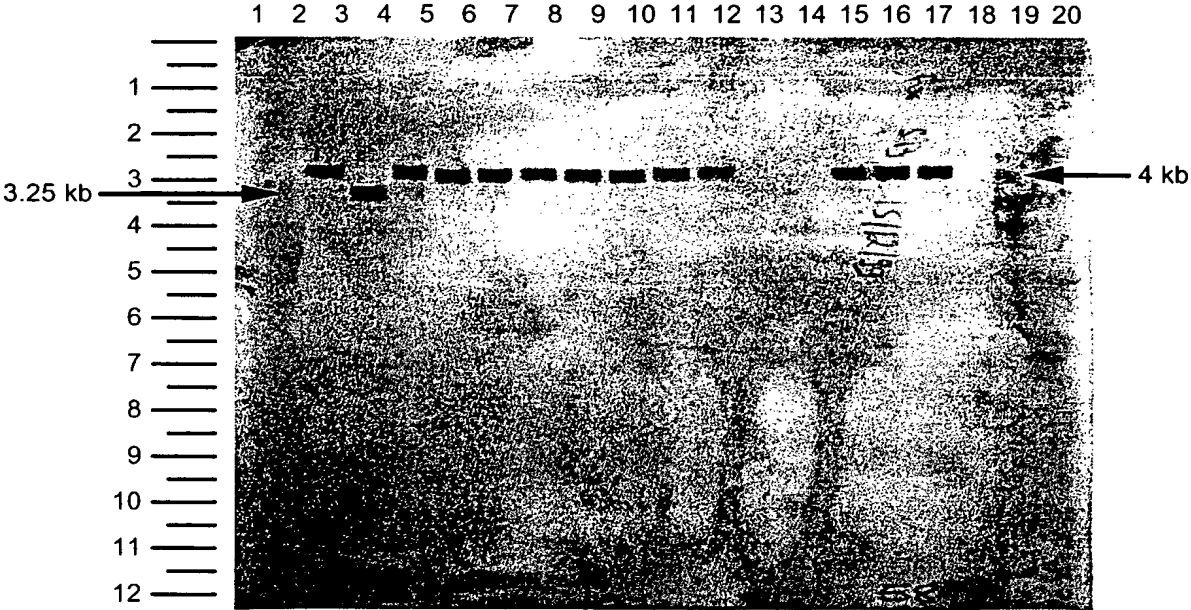


FIG. 14

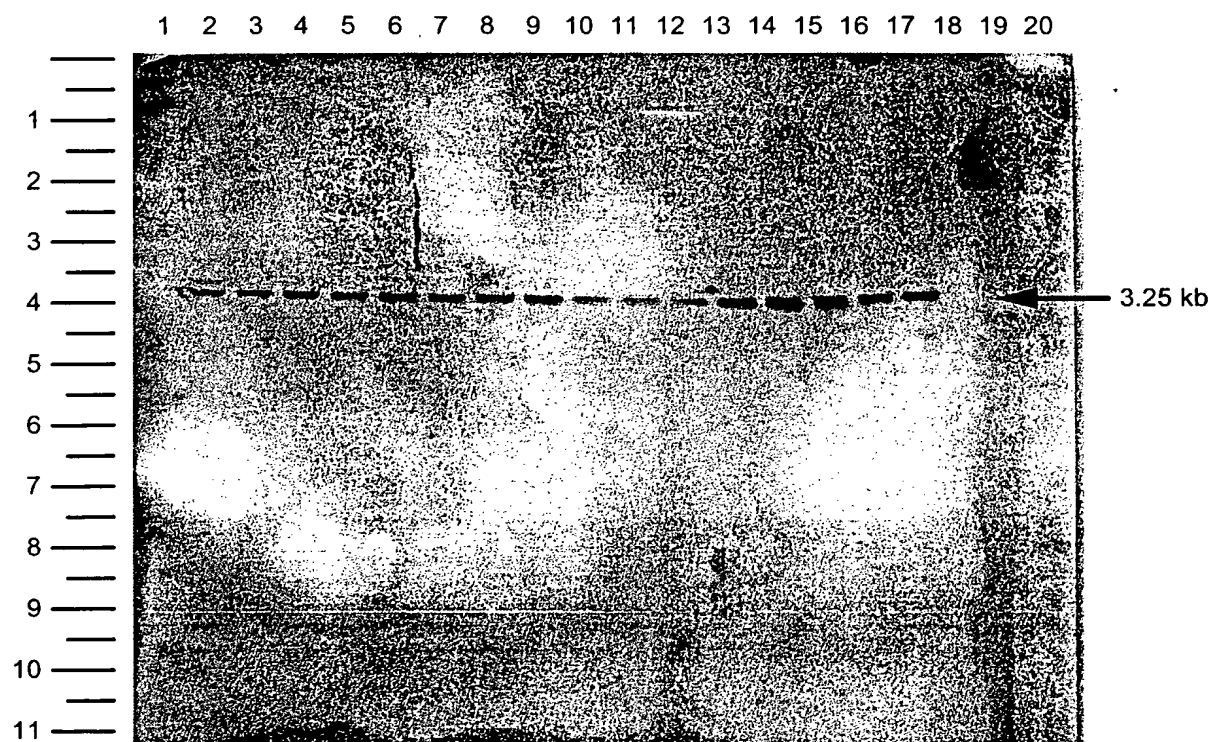
Southern blot analysis - ID-89



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# FIG. 15

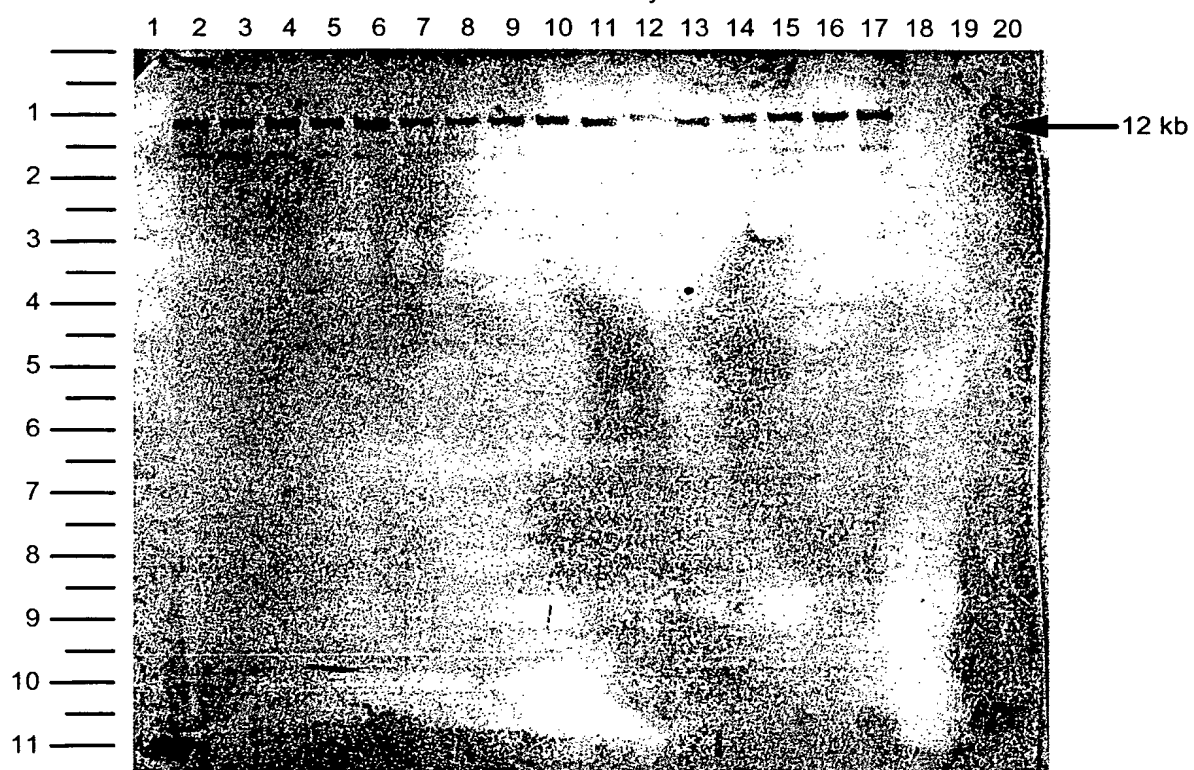
Southern blot analysis - ID-93



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**FIG. 16**

Southern blot analysis - ID-96



(19) World Intellectual Property Organization  
International Bureau



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10 May 2001 (10.05.2001)

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39/09, 48/00, G01N 33/53, 33/68

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For two-letter codes and other abbreviations, refer to the "Guid-  
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ning of each regular issue of the PCT Gazette.

(54) Title: NUCLEIC ACIDS AND PROTEINS FROM GROUP B STREPTOCOCCUS

(57) Abstract: Novel protein antigens from Group B Streptococcus are described, together with the nucleic acid sequences encoding them. The use of vaccines and screening methods is also described.

WO 01/32882 A3



# INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 00/03437

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/31 C12Q1/68 C12N1/21 C07K14/315 C07K16/12  
A61K39/09 A61K48/00 G01N33/53 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K C12N C12Q A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

BIOSIS, EPO-Internal, EMBL

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No. |
|------------|---|-----------------------|
| X          | WO 97 08553 A (UNIV CALIFORNIA)<br>6 March 1997 (1997-03-06)<br>Seq Id No: 4<br>page 5, line 25 - line 30<br>claim 2<br>---<br>-/-- | 1-16,<br>21-23        |



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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"P" document published prior to the international filing date but later than the priority date claimed

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

28 May 2001

Date of mailing of the international search report

12. 06. 2001

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Authorized officer

van Klompenburg, W

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 00/03437

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No. |
|------------|---|-----------------------|
| X          | <p>SANCHEZ-BEATO: "Molecular characterization of PcpA: a novel choline-binding protein of Streptococcus pneumoniae"<br/> FEMS MICROBIOL LETT,<br/> vol. 164, no. 1, 1 July 1998 (1998-07-01),<br/> pages 207-214, XP000992682<br/> page 213, left-hand column; figures 1,2<br/> -&amp; DATABASE EMBL 'Online!<br/> ebi;<br/> ACC. NO.: z82001,<br/> 30 March 1997 (1997-03-30)<br/> SANCHEZ-BEATO ET AL.: "S. pneumoniae pcpA gene and open reading frame"<br/> XP002168347<br/> abstract</p> | 1-16,<br>21-23        |
| A          | <p>MICHEL J L ET AL: "Cloned alpha and beta C-protein antigens of group B Streptococci elicit protective immunity"<br/> INFECTION AND IMMUNITY,US,AMERICAN SOCIETY FOR MICROBIOLOGY. WASHINGTON,<br/> vol. 59, no. 6, June 1991 (1991-06), pages<br/> 2023-2028, XP002107260<br/> ISSN: 0019-9567<br/> figures 1,2,5</p>  | 1-24                  |
| A          | <p>WO 99 16882 A (MEDIMMUNE INC)<br/> 8 April 1999 (1999-04-08)<br/> claims 1-12,17; figures 1-8</p>  | 1-24                  |
| A          | <p>LARSSON C ET AL: "Experimental vaccination against group B Streptococcus, an encapsulated bacterium, with highly purified preparations of cell surface proteins Rib and alfa"<br/> INFECTION AND IMMUNITY,US,AMERICAN SOCIETY FOR MICROBIOLOGY. WASHINGTON,<br/> vol. 63, no. 9, September 1996 (1996-09),<br/> pages 3518-3523, XP002109333<br/> ISSN: 0019-9567<br/> figures 1-3; tables 1,2</p>   | 1-24                  |
| A          | <p>US 5 928 900 A (MASURE H ROBERT ET AL)<br/> 27 July 1999 (1999-07-27)<br/> figures 1B,2-17,19,22; examples 1-4</p>   | 1-24                  |

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/GB 00/03437

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☒ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:  
1-24 all partially
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☒ No protest accompanied the payment of additional search fees.

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: Invention 1, claims 1-24 all partially

A Streptococcus agalactiae protein or polypeptide having a sequence as depicted in SeqIdNo.2; a homologue or derivative of said protein or polypeptide; an antigenic and/or immunogenic fragment of said protein or polypeptide; a nucleic acid molecule comprising or consisting of SeqIdNo.1, a nucleic acid molecule complementary to said sequence, a nucleic acid molecule encoding for the same or a homologue, derivative or fragment of said protein or polypeptide; use of said protein or polypeptide as an immunogen and/or an antigen; an immunogenic composition and/or antigenic composition comprising said protein or polypeptide; an antibody to said protein or polypeptide; a method of detection/diagnosis of S.pneumoniae comprising using said protein or polypeptide, said antibody, or said nucleic acid molecule; a kit for the detection of S. galactiae comprising said protein, polypeptide, antibody or nucleic acid; a method of determining whether said protein or polypeptide represents a potential antimicrobial target which comprises inactivating said protein or polypeptide and determining whether S. agalactiae is still viable.

2. Claims: Inventions 2-122, claims 1-24 all partially

Idem as subject 1 but limited to each of the polynucleotide and polypeptide sequences as in SeqIdNo:3-244, wherein invention 2 is limited to SeqIdNo:3 and SeqIdNo:4, invention 3 is limited to SeqIdNo:5 and SeqIdNo:6, ..., invention 122 is limited to SeqIdNo:243 and 244,

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 00/03437

| Patent document<br>cited in search report |   | Publication<br>date | Patent family<br>member(s) |           | Publication<br>date |
|---|---|---------------------|----------------------------|-----------|---------------------|
| WO 9708553                                | A | 06-03-1997          | AU                         | 6913396 A | 19-03-1997          |
| WO 9916882                                | A | 08-04-1999          | AU                         | 9507698 A | 23-04-1999          |
|   |   |                     | EP                         | 1037997 A | 27-09-2000          |
| US 5928900                                | A | 27-07-1999          | AU                         | 709405 B  | 26-08-1999          |
|   |   |                     | AU                         | 7680994 A | 22-03-1995          |
|   |   |                     | CA                         | 2170726 A | 09-03-1995          |
|   |   |                     | EP                         | 0721506 A | 17-07-1996          |
|   |   |                     | FI                         | 960977 A  | 30-04-1996          |
|   |   |                     | JP                         | 9504686 T | 13-05-1997          |
|   |   |                     | NO                         | 960839 A  | 19-04-1996          |
|   |   |                     | NZ                         | 273497 A  | 25-03-1998          |
|   |   |                     | WO                         | 9506732 A | 09-03-1995          |
|   |   |                     | US                         | 5981229 A | 09-11-1999          |